```
=> e cole stewart/au
E1            1     COLE STEVEN ROGER/AU
E2           29     COLE STEVEN W/AU
E3           48 --> COLE STEWART/AU
E4          412     COLE STEWART T/AU
E5            1     COLE STEWART THOMAS/AU
E6            2     COLE STORRS W/AU
E7           18     COLE STRAUSS A/AU
E8            1     COLE STRAUSS A C/AU
E9            1     COLE STRAUSS A D/AU
E10          25     COLE STRAUSS ALLYSON/AU
E11           4     COLE STRAUSS ALLYSON D/AU
E12           3     COLE STUART J/AU

=> s e3-e5 and (BCG or microti) and (RD1-2F9)
L1            6 ("COLE STEWART"/AU OR "COLE STEWART T"/AU OR "COLE STEWART THOMA
                S"/AU) AND (BCG OR MICROTI) AND (RD1-2F9)

=> dup rem l1
PROCESSING COMPLETED FOR L1
L2            2 DUP REM L1 (4 DUPLICATES REMOVED)

=> d bib ab 1-
YOU HAVE REQUESTED DATA FROM 2 ANSWERS - CONTINUE? Y/(N):y

L2   ANSWER 1 OF 2  BIOSIS  COPYRIGHT (c) 2007 The Thomson Corporation  on STN
     DUPLICATE 1
AN   2004:326990  BIOSIS
DN   PREV200400328635
TI   Enhanced protection against tuberculosis by vaccination with recombinant
     Mycobacterium microti vaccine that induces T cell immunity
     against region of difference 1 antigens.
AU   Brodin, Priscille; Majlessi, Laleh; Brosch, Roland; Smith, Debbie;
     Bancroft, Gregory; Clark, Simon; Williams, Ann; Leclerc, Claude;
     Cole, Stewart T. [Reprint Author]
CS   Unite Genet Mol Bacterienne, Inst Pasteur, 28 Rue Docteur Roux, F-75724,
     Paris, 15, France
     stcole@pasteur.fr
SO   Journal of Infectious Diseases, (July 1 2004) Vol. 190, No. 1, pp.
     115-122. print.
     CODEN: JIDIAQ. ISSN: 0022-1899.
DT   Article
LA   English
ED   Entered STN: 29 Jul 2004
     Last Updated on STN: 29 Jul 2004
AB   Mycobacterium microti, the vole bacillus, which was used as a
     live vaccine against tuberculosis until the 1970s, confers the same
     protection in humans as does Mycobacterium bovis bacille Calmette-Guerin (
     BCG).  However, because the efficacy of the BCG vaccine
     varies considerably, we have tried to develop a better vaccine by
     reintroducing into M. microti the complete region of difference
     1 (RD1), which is required for secretion of the potent T cell antigens
     early secreted antigen target (ESAT)-6 and culture filtrate protein
     (CFP)-10.  The resultant recombinant strain, M. microti OV254::
     RD1-2F9, induced specific ESAT-6 and CFP-10 immune
     responses in mice with CD8+ T lymphocytes that had strong expression of
     the CD44hi activation marker.  This vaccine also displayed better efficacy
     against disseminated disease in the mouse and the guinea pig models of
     tuberculosis than was seen in animals vaccinated with M. microti
     alone or with BCG.  The M. microti OV254::RD1
     -2F9 vaccine was less virulent and persistent in mice and than
     was BCG::RD1-2F9 may represent a safer
     alternative to BCG::RD1-2F9.
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L2    ANSWER 2 OF 2  BIOSIS  COPYRIGHT (c) 2007 The Thomson Corporation  on STN
      DUPLICATE 2
AN    2003:249243  BIOSIS
DN    PREV200300249243
TI    Recombinant BCG exporting ESAT-6 confers enhanced protection
      against tuberculosis.
AU    Pym, Alexander S.; Brodin, Priscille; Majlessi, Laleh; Brosch, Roland;
      Demangel, Caroline; Williams, Ann; Griffiths, Karen E.; Marchal, Gilles;
      Leclerc, Claude; Cole, Stewart T. [Reprint Author]
CS    Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, Paris,
      France
      stcole@pasteur.fr
SO    Nature Medicine, (May 2003) Vol. 9, No. 5, pp. 533-539. print.
      ISSN: 1078-8956 (ISSN print).
DT    Article
LA    English
ED    Entered STN: 28 May 2003
      Last Updated on STN: 28 May 2003
AB    The live tuberculosis vaccines Mycobacterium bovis BCG (bacille
      Calmette-Guerin) and Mycobacterium microti both lack the potent,
      secreted T-cell antigens ESAT-6 (6-kDa early secretory antigenic target)
      and CFP-10 (10-kDa culture filtrate protein).  This is a result of
      independent deletions in the region of deletion-1 (RD1) locus, which is
      intact in virulent members of the Mycobacterium tuberculosis complex.  To
      increase their immunogenicity and protective capacity, we complemented
      both vaccines with different constructs containing the esxA and esxB
      genes, which encode ESAT-6 and CFP-10 respectively, as well as a variable
      number of flanking genes.  Only reintroduction of the complete locus,
      comprising at least 11 genes, led to full secretion of the antigens and
      resulted in specific ESAT-6-dependent immune responses; this suggests that
      the flanking genes encode a secretory apparatus.  Mice and guinea pigs
      vaccinated with the recombinant strain BCG::RD1-
      2F9 were better protected against challenge with M. tuberculosis,
      showing less severe pathology and reduced dissemination of the pathogen,
      as compared with control animals immunized with BCG alone.


=> e pym alexander s/au
E1            5      PYM ALEX S/AU
E2            5      PYM ALEXANDER/AU
E3           34 --> PYM ALEXANDER S/AU
E4           66      PYM B/AU
E5            5      PYM B A/AU
E6            3      PYM B M/AU
E7            2      PYM BARBARA/AU
E8            2      PYM BARBARA A/AU
E9            1      PYM C/AU
E10           3      PYM C A/AU
E11           4      PYM CAROLINE/AU
E12           2      PYM D P/AU

=> s e1-e3 and (bcg or microti)
L3           19 ("PYM ALEX S"/AU OR "PYM ALEXANDER"/AU OR "PYM ALEXANDER S"/AU)
                AND (BCG OR MICROTI)

=> dup rem l3
PROCESSING COMPLETED FOR L3
L4             9 DUP REM L3 (10 DUPLICATES REMOVED)

=> d bib ab 1-
YOU HAVE REQUESTED DATA FROM 9 ANSWERS - CONTINUE? Y/(N):y

L4    ANSWER 1 OF 9  BIOSIS  COPYRIGHT (c) 2007 The Thomson Corporation  on STN
```

DUPLICATE 1
AN 2005:205775 BIOSIS
DN PREV200500205883
TI Evaluation of vaccines in the EU TB vaccine cluster using a guinea pig aerosol infection model of tuberculosis.
AU Williams, Ann [Reprint Author]; Hatch, Graham J.; Clark, Simon O.; Gooch, Karen E.; Hatch, Kim A.; Hall, Graham A.; Huygen, Kris; Ottenhoff, Tom H. M.; Franken, Kees L. M. C.; Andersen, Peter; Doherty, T. Mark; Kaufmann, Stefan H. E.; Grode, Leander; Seiler, Peter; Martin, Carlos; Gicquel, Brigitte; Cole, Stewart T.; Brodin, Priscille; Pym, Alexander S. ; Dalemans, Wilfried; Cohen, Joe; Lobet, Yves; Goonetilleke, Nilu; McShane, Helen; Hill, Adrian; Parish, Tanya; Smith, Debbie; Stoker, Neil G.; Lowrie, Douglas B.; Kallenius, Gunilla; Svenson, Stefan; Pawowski, Andrzej; Blake, Karen; Marsh, Philip D.
CS Hlth Protect Agcy, Porton Down, Salisbury, Wilts, SP4 0JG, UK
ann.williams@camr.org.uk
SO Tuberculosis (Amsterdam), (January 2005) Vol. 85, No. 1-2, pp. 29-38. print.
ISSN: 1472-9792 (ISSN print).
DT Article
LA English
ED Entered STN: 1 Jun 2005
Last Updated on STN: 1 Jun 2005
AB The TB Vaccine Cluster project funded by the EU Fifth Framework programme aims to provide novel vaccines against tuberculosis that are suitable for evaluation in humans. This paper describes the studies of the protective efficacy of vaccines in a guinea pig aerosol-infection model of primary tuberculosis. The objective was to conduct comparative evaluations of vaccines that had previously demonstrated efficacy in other animal models. Groups of 6 guinea pigs were immunized with vaccines provided by the relevant EU Vaccine Cluster partners. Survival over 17 or 26 weeks was used as the principal measure of vaccine efficacy following aerosol challenge with H37Rv. Counts of mycobacteria in lungs and spleens, and histopathological changes in the lungs, were also used to provide evidence of protection. A total of 24 vaccines were evaluated in 4 experiments each of a different design. A heterologous prime-boost strategy of DNA and MVA, each expressing Ag85A and a fusion protein of ESAT-6 and Ag85B in adjuvant, protected the guinea pigs to the same extent as BCG. Genetically modified BCG vaccines and boosted BCG strategies also protected guinea pigs to the same extent as BCG but not statistically significantly better. A relatively high aerosol-challenge dose and evaluation over a protracted time post-challenge allowed superior protection over BCG to be demonstrated by BCG boosted with MVA and fowl pox vectors expressing Ag85A.

L4 ANSWER 2 OF 9 CAPLUS
AN 2003:791413 CAPLUS
DN 139:304478
TI Identification of virulence associated regions RD1 and RD5 enabling the development of improved vaccines of M. bovis BCG and M. microti
IN Cole, Stewart; Pym, Alexander S.; Brosch, Roland; Brodin, Priscille; Majlessi, Laleh; Leclerc, Claude
PA Institut Pasteur, Fr.
SO Eur. Pat. Appl., 58 pp.
CODEN: EPXXDW
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI EP 1350839	A1	20031008	EP 2002-290864	20020405
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,				

IE, SI, LT, LV, FI, RO, MK, CY, AL, TR
CA 2481318 A1 20031016 CA 2003-2481318 20030401
WO 2003085098 A2 20031016 WO 2003-IB1789 20030401
WO 2003085098 A3 20040129
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
AU 2003223039 A1 20031020 AU 2003-223039 20030401
EP 1492867 A2 20050105 EP 2003-719008 20030401
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK
US 2005220811 A1 20051006 US 2004-510021 20041001
PRAI EP 2002-290864 A 20020405
WO 2003-IB1789 W 20030401

AB The present invention relates to a strain of Mycobacterium bovis BCG or Mycobacterium microti, wherein said strain has integrated part or all of the RD1 region responsible for enhanced immunogenicity of the tubercle bacilli, especially the ESAT-6 and CFP-10 genes. These strains will be referred as the M. bovis BCG::RD1 or M. microti::RD1 strains and are useful as a new improved vaccine for preventing tuberculosis and as a therapeutical product enhancing the stimulation of the immune system for the treatment of bladder cancer.

RE.CNT 11 THERE ARE 11 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 3 OF 9 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 2
AN 2003:249243 BIOSIS
DN PREV200300249243
TI Recombinant BCG exporting ESAT-6 confers enhanced protection against tuberculosis.
AU Pym, Alexander S.; Brodin, Priscille; Majlessi, Laleh; Brosch, Roland; Demangel, Caroline; Williams, Ann; Griffiths, Karen E.; Marchal, Gilles; Leclerc, Claude; Cole, Stewart T. [Reprint Author]
CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, Paris, France
stcole@pasteur.fr
SO Nature Medicine, (May 2003) Vol. 9, No. 5, pp. 533-539. print.
ISSN: 1078-8956 (ISSN print).
DT Article
LA English
ED Entered STN: 28 May 2003
Last Updated on STN: 28 May 2003
AB The live tuberculosis vaccines Mycobacterium bovis BCG (bacille Calmette-Guerin) and Mycobacterium microti both lack the potent, secreted T-cell antigens ESAT-6 (6-kDa early secretory antigenic target) and CFP-10 (10-kDa culture filtrate protein). This is a result of independent deletions in the region of deletion-1 (RD1) locus, which is intact in virulent members of the Mycobacterium tuberculosis complex. To increase their immunogenicity and protective capacity, we complemented both vaccines with different constructs containing the esxA and esxB genes, which encode ESAT-6 and CFP-10 respectively, as well as a variable number of flanking genes. Only reintroduction of the complete locus, comprising at least 11 genes, led to full secretion of the antigens and resulted in specific ESAT-6-dependent immune responses; this suggests that the flanking genes encode a secretory apparatus. Mice and guinea pigs vaccinated with the recombinant strain BCG::RD1-2F9 were better

protected against challenge with M. tuberculosis, showing less severe pathology and reduced dissemination of the pathogen, as compared with control animals immunized with BCG alone.

L4 ANSWER 4 OF 9 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 3
AN 2002:614464 BIOSIS
DN PREV200200614464
TI Loss of RD1 contributed to the attenuation of the live tuberculosis vaccines Mycobacterium bovis BCG and Mycobacterium microti.
AU Pym, Alexander S.; Brodin, Priscille; Brosch, Roland; Huerre, Michel; Cole, Stewart T. [Reprint author]
CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, 28 Rue du Docteur Roux, 75724, Paris Cedex 15, France
stcole@pasteur.fr
SO Molecular Microbiology, (November, 2002) Vol. 46, No. 3, pp. 709-717. print.
CODEN: MOMIEE. ISSN: 0950-382X.
DT Article
LA English
ED Entered STN: 4 Dec 2002
Last Updated on STN: 4 Dec 2002
AB Although large human populations have been safely immunized against tuberculosis with two live vaccines, Mycobacterium bovis BCG or Mycobacterium microti, the vole bacillus, the molecular basis for the avirulence of these vaccine strains remains unknown. Comparative genomics has identified a series of chromosomal deletions common to both virulent and avirulent species but only a single locus, RD1, that has been deleted from M. bovis BCG and M. microti. Restoration of RD1, by gene knock-in, resulted in a marked change in colonial morphology towards that of virulent tubercle bacilli. Three RD1-encoded proteins were localized in the cell wall, and two of them, the immunodominant T-cell antigens ESAT-6 and CFP-10, were also found in culture supernatants. The BCG::RD1 and M. microti ::RD1 knock-ins grew more vigorously than controls in immunodeficient mice, inducing extensive splenomegaly and granuloma formation. Increased persistence and partial reversal of attenuation were observed when immunocompetent mice were infected with the BCG::RD1 knock-in, whereas BCG controls were cleared. Knocking-in five other RD loci did not affect the virulence of BCG. This study describes a genetic lesion that contributes to safety and opens new avenues for vaccine development.

L4 ANSWER 5 OF 9 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 4
AN 2003:103124 BIOSIS
DN PREV200300103124
TI Proteome analysis of the plasma membrane of Mycobacterium tuberculosis.
AU Sinha, Sudhir [Reprint Author]; Arora, Shalini; Kosalai, K.; Namane, Abdelkader; Pym, Alex S.; Cole, Stewart T.
CS Division of Biochemistry, Central Drug Research Institute, PO Box 173, Lucknow, 226001, India
sinhas@lycos.com
SO Comparative and Functional Genomics, (December 2002) Vol. 3, No. 6, pp. 470-483. print.
ISSN: 1531-6912 (ISSN print).
DT Article
LA English
ED Entered STN: 19 Feb 2003
Last Updated on STN: 19 Feb 2003
AB The plasma membrane of Mycobacterium tuberculosis is likely to contain proteins that could serve as novel drug targets, diagnostic probes or even components of a vaccine against tuberculosis. With this in mind, we have

undertaken proteome analysis of the membrane of M. tuberculosis H37Rv. Isolated membrane vesicles were extracted with either a detergent (Triton X114) or an alkaline buffer (carbonate) following two of the protocols recommended for membrane protein enrichment. Proteins were resolved by 2D-GE using immobilized pH gradient (IPG) strips, and identified by peptide mass mapping utilizing the M. tuberculosis genome database. The two extraction procedures yielded patterns with minimal overlap. Only two proteins, both HSPs, showed a common presence. MALDI-MS analysis of 61 spots led to the identification of 32 proteins, 17 of which were new to the M. tuberculosis proteome database. We classified 19 of the identified proteins as 'membrane-associated'; 14 of these were further classified as 'membrane-bound', three of which were lipoproteins. The remaining proteins included four heat-shock proteins and several enzymes involved in energy or lipid metabolism. Extraction with Triton X114 was found to be more effective than carbonate for detecting 'putative' M. tuberculosis membrane proteins. The protocol was also found to be suitable for comparing BCG and M. tuberculosis membranes, identifying ESAT-6 as being expressed selectively in M. tuberculosis. While this study demonstrates for the first time some of the membrane proteins of M. tuberculosis, it also underscores the problems associated with proteomic analysis of a complex membrane such as that of a mycobacterium.

L4 ANSWER 6 OF 9 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 5
AN 2001:465041 BIOSIS
DN PREV200100465041
TI The evolution of mycobacterial pathogenicity: Clues from comparative genomics.
AU Brosch, Roland [Reprint author]; Pym, Alexander S. [Reprint author]; Gordon, Stephen V.; Cole, Stewart T. [Reprint author]
CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, 28 Rue du Dr Roux, 75724, Paris Cedex 15, France
stcole@pasteur.fr
SO Trends in Microbiology, (September, 2001) Vol. 9, No. 9, pp. 452-458. print.
ISSN: 0966-842X.
DT Article
General Review; (Literature Review)
LA English
ED Entered STN: 3 Oct 2001
Last Updated on STN: 23 Feb 2002
AB Comparative genomics, and related technologies, are helping to unravel the molecular basis of the pathogenesis, host range, evolution and phenotypic differences of the slow-growing mycobacteria. In the highly conserved Mycobacterium tuberculosis complex, where single-nucleotide polymorphisms are rare, insertion and deletion events (InDels) are the principal source of genome plasticity. InDels result from recombinational or insertion sequence (IS)-mediated events, expansion of repetitive DNA sequences, or replication errors based on repetitive motifs that remove blocks of genes or contract coding sequences. Comparative genomic analyses also suggest that loss of genes is part of the ongoing evolution of the slow-growing mycobacterial pathogens and might also explain how the vaccine strain BCG became attenuated.

L4 ANSWER 7 OF 9 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 6
AN 2001:49988 BIOSIS
DN PREV200100049988
TI Tools for the population genomics of the tubercle bacilli.
AU Pym, Alexander S.; Brosch, Roland [Reprint author]
CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, 75724, Paris Cedex 15, France
rbrosch@pasteur.fr
SO Genome Research, (December, 2000) Vol. 10, No. 12, pp. 1837-1839. print.

ISSN: 1088-9051.
DT Article
LA English
ED Entered STN: 24 Jan 2001
Last Updated on STN: 12 Feb 2002

L4 ANSWER 8 OF 9 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
AN 2000:430291 BIOSIS
DN PREV200000430291
TI Comparative genomics of the mycobacteria.
AU Brosch, Roland [Reprint author]; Gordon, Stephen V.; Pym, Alexander; Eiglmeier, Karin; Garnier, Thierry; Cole, Stewart T.
CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, 28 rue du Docteur Roux, 75724, Paris Cedex, 15, France
SO IJMM International Journal of Medical Microbiology, (May, 2000) Vol. 290, No. 2, pp. 143-152. print.
ISSN: 1438-4221.
DT Article
General Review; (Literature Review)
LA English
ED Entered STN: 11 Oct 2000
Last Updated on STN: 10 Jan 2002
AB The genus mycobacteria includes two important human pathogens Mycobacterium tuberculosis and Mycobacterium lepra. The former is reputed to have the highest annual global mortality of all pathogens. Their slow growth, virulence for humans and particular physiology makes these organisms extremely difficult to work with. However the rapid development of mycobacterial genomics following the completion of the Mycobacterium tuberculosis genome sequence provides the basis for a powerful new approach for the understanding of these organisms. Five further genome sequencing projects of closely related mycobacterial species with differing host range, virulence for humans and physiology are underway. A comparative genomic analysis of these species has the potential to define the genetic basis of these phenotypes which will be invaluable for the development of urgently needed new vaccines and drugs. This minireview summarises the different techniques that have been employed to compare these genomes and gives an overview of the wealth of data that has already been generated by mycobacterial comparative genomics.

L4 ANSWER 9 OF 9 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 7
AN 2000:418561 BIOSIS
DN PREV200000418561
TI Comparative genomics uncovers large tandem chromosomal duplications in Mycobacterium bovis BCG pasteur.
AU Brosch, Roland; Gordon, Stephen V.; Buchrieser, Carmen; Pym, Alexander S.; Garnier, Thierry; Cole, Stewart T. [Reprint author]
CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, 28 Rue du Dr Roux, 75724, Paris Cedex, 15, France
SO Yeast, (30 June, 2000) Vol. 17, No. 2, pp. 111-123. print.
CODEN: YESTE3. ISSN: 0749-503X.
DT Article
LA English
ED Entered STN: 4 Oct 2000
Last Updated on STN: 8 Jan 2002
AB On direct comparison of minimal sets of ordered clones from bacterial artificial chromosome (BAC) libraries representing the complete genomes of Mycobacterium tuberculosis H37Rv and the vaccine strain, Mycobacterium bovis BCG Pasteur, two major rearrangements were identified in the genome of M. bovis BCG Pasteur. These were shown to correspond to two tandem duplications, DU1 and DU2, of 29 668 bp and 36 161 bp, respectively. While DU1 resulted from a single duplication event, DU2 apparently arose from duplication of a 100 kb genomic segment that subsequently incurred an internal deletion of 64 kb. Several lines of

evidence suggest that DU2 may continue to expand, since two copies were detected in a subpopulation of BCG Pasteur cells. BCG strains harbouring DU1 and DU2 are diploid for at least 58 genes and contain two copies of oriC, the chromosomal origin of replication. These findings indicate that these genomic regions of the BCG genome are still dynamic. Although the role of DU1 and DU2 in the attenuation and/or altered immunogenicity of BCG is yet unknown, knowledge of their existence will facilitate quality control of BCG vaccine lots and may help in monitoring the efficacy of the world's most widely used vaccine.

```
=> e brosch roland/au
E1            1     BROSCH REGINA/AU
E2            1     BROSCH RICHARD WILLIAM/AU
E3          120 --> BROSCH ROLAND/AU
E4            3     BROSCH ROLLAND/AU
E5            3     BROSCH RUDOLF/AU
E6           35     BROSCH S/AU
E7            7     BROSCH S F/AU
E8            3     BROSCH SABINE/AU
E9            4     BROSCH SALOMON S/AU
E10           3     BROSCH SALOMON SUSANNE/AU
E11          13     BROSCH SIBYLLE/AU
E12           1     BROSCH STARZENGRUBER NORBERT/AU

=> s e3-e4 and (bcg or microti)
L5           58 ("BROSCH ROLAND"/AU OR "BROSCH ROLLAND"/AU) AND (BCG OR MICROTI)


=> dup rem l5
PROCESSING COMPLETED FOR L5
L6            23 DUP REM L5 (35 DUPLICATES REMOVED)

=> d bib ab 1-
YOU HAVE REQUESTED DATA FROM 23 ANSWERS - CONTINUE? Y/(N):y


L6   ANSWER 1 OF 23  CAPLUS  COPYRIGHT 2007 ACS on STN
AN   2007:41383  CAPLUS
DN   146:140994
TI   Modified ESAT-6 derived from Mycobacterium tuberculosis and Mycobacterium
     leprae as vaccines for inducing interferon γ response to ESAT-6
     and/or CFP-10 against infection
IN   Brosch, Roland; Brodin, Priscille; Cole, Stewart; Majlessi,
     Laleh; Leclerc, Claude
PA   Fr.
SO   U.S. Pat. Appl. Publ., 64pp.
     CODEN: USXXCO
DT   Patent
LA   English
FAN.CNT 1
     PATENT NO.          KIND   DATE        APPLICATION NO.         DATE
     --------------      ----   --------    --------------------    --------
PI   US 2007009547        A1    20070111    US 2006-455929.         20060620
     WO 2007010413        A2    20070125    WO 2006-IB2884          20060622
     WO 2007010413        A3    20070830
          W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH,
              CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD,
              GE, GH, GM, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP,
              KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN,
              MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU,
              SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG,
              US, UZ, VC, VN, ZA, ZM, ZW
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RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AP, EA, EP, OA

PRAI US 2005-692561P P 20050622

AB A genetically modified strain of M. tuberculosis or Mycobacterium bovis BCG is provided, wherein the genetically modified strain comprises at least one modified sequence comprising SEQ ID NO: 1, SEQ ID NO: 2, or both, having at least one mutation at T2, Q4, F8, A14, L28, L29, W43, G45, Q55, Q56, N66, M83, V90, M93, or F94. In a preferred embodiment, the mutation is at least one of T2H, Q4L, F8I, A14R, L28A, L29S, W43R, G45T, Y51, Q55I, Q56A, N66I, N66A, M83I, V90R, M93T, or F94Q. Similarly, the genetically modified strain may also secrete ESAT-6 with a a histidine tag, tetra-cysteine tag or FLAG-tag, a GFP-fusion, or a short truncation at the C-terminal end of less than 20 amino acids.

L6 ANSWER 2 OF 23 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 1

AN 2007:313634 BIOSIS

DN PREV200700314718

TI Genome plasticity of BCG and impact on vaccine efficacy.

AU Brosch, Roland; Gordon, Stephen V.; Garnier, Thierry; Eiglmeier, Karin; Frigui, Wafa; Valenti, Philippe; Dos Santos, Sandrine; Duthoy, Stephanie; Lacroix, Celine; Garcia-Pelayo, Carmen; Inwald, Jacqueline K.; Golby, Paul; Garcia, Javier Nunez; Hewinson, R. Glyn; Behr, Marcel A.; Quail, Michael A.; Churcher, Carol; Barrell, Bart G.; Parkhill, Julian; Cole, Stewart T. [Reprint Author]

CS Inst Pasteur, Unite Genet Mol Bacterienne, 28 Rue Dr Roux, F-75724 Paris, France
stcole@pasteur.fr

SO Proceedings of the National Academy of Sciences of the United States of America, (MAR 27 2007) Vol. 104, No. 13, pp. 5596-5601.
CODEN: PNASA6. ISSN: 0027-8424.

DT Article

LA English

OS GenBank-AM408590; EMBL-AM408590; DDBJ-AM408590

ED Entered STN: 16 May 2007
Last Updated on STN: 16 May 2007

AB To understand the evolution, attenuation, and variable protective efficacy of bacillus Calmette-Guerin (BCG) vaccines, Mycobacterium bovis BCG Pasteur 1173P2 has been subjected to comparative genome and transcriptome analysis. The 4,374,522-by genome contains 3,954 protein-coding genes, 58 of which are present in two copies as a result of two independent tandem duplications, DU1 and DU2. DU1 is restricted to BCG Pasteur, although four forms of DU2 exist; DU2-I is confined to early BCG vaccines, like BCG Japan, whereas DU2-III and DU2-IV occur in the late vaccines. The glycerol-3-phosphate dehydrogenase gene, glpD2, is one of only three genes common to all four DU2 variants, implying that BCG requires higher levels of this enzyme to grow on glycerol. Further amplification of the DU2 region is ongoing, even within vaccine preparations used to immunize humans. An evolutionary scheme for BCG vaccines was established by analyzing DU2 and other markers. Lesions in genes encoding a-factors and pleiotropic transcriptional regulators, like PhoR and Crp, were also uncovered in various BCG strains; together with gene amplification, these affect gene expression levels, immunogenicity, and, possibly, protection against tuberculosis. Furthermore, the combined findings suggest that early BCG vaccines may even be superior to the later ones that are more widely used.

L6 ANSWER 3 OF 23 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 2

AN 2006:176683 BIOSIS

DN PREV200600166449
TI Dissection of ESAT-6 system 1 of Mycobacterium tuberculosis and impact on immunogenicity and virulence.
AU Brodin, Priscille; Majlessi, Laleh; Marsollier, Laurent; de Jonge, Marien I.; Bottai, Daria; Demangel, Caroline; Hinds, Jason; Neyrolles, Olivier; Butcher, Philip D.; Leclerc, Claude; Cole, Stewart T.; Brosch, Roland [Reprint Author]
CS Inst Pasteur, Unite Genet Mol Bacterienne, 25-28 Rue Docteur Roux, F-75724 Paris 15, France
rbrosch@pasteur.fr
SO Infection and Immunity, (JAN 2006) Vol. 74, No. 1, pp. 88-98.
CODEN: INFIBR. ISSN: 0019-9567.
DT Article
LA English
ED Entered STN: 9 Mar 2006
Last Updated on STN: 9 Mar 2006
AB The dedicated secretion system ESX-1 of Mycobacterium tuberculosis encoded by the extended RD1 region (extRD1) assures export of the ESAT-6 protein and its partner, the 10-kDa culture filtrate protein CFP-10, and is missing from the vaccine strains M. bovis BCG and M. microti. Here, we systematically investigated the involvement of each individual ESX-1 gene in the secretion of both antigens, specific immunogenicity, and virulence. ESX-1-complemented BCG and M. microti strains were more efficiently engulfed by bone-marrow-derived macrophages than controls, and this may account for the enhanced in vivo growth of ESX-1-carrying strains. Inactivation of gene pe35 (Rv3872) impaired expression of CFP-10 and ESAT-6, suggesting a role in regulation. Genes Rv3868, Rv3869, Rv3870, Rv3871, and Rv3877 encoding an ATP-dependent chaperone and translocon were essential for secretion of ESAT-6 and CFP-10 in contrast to ppe68 Rv3873 and Rv3876, whose inactivation did not impair secretion of ESAT-6. A strict correlation was found between ESAT-6 export and the generation of ESAT-6 specific T-cell responses in mice. Furthermore, ESAT-6 secretion and specific immunogenicity were almost always correlated with enhanced virulence in the SCID mouse model. Only loss of Rv3865 and part of Rv3866 did not affect ESAT-6 secretion or immunogenicity but led to attenuation. This suggests that Rv3865/66 represent a new virulence factor that is independent from ESAT-6 secretion. The present study has allowed us to identify new aspects of the extRD1 region of M. tuberculosis and to explore its role in the pathogenesis of tuberculosis.

L6 ANSWER 4 OF 23 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 3
AN 2006:7638 BIOSIS
DN PREV200600007409
TI Functional analysis of early secreted antigenic target-6, the dominant T-cell antigen of Mycobacterium tuberculosis, reveals key residues involved in secretion, complex formation, virulence, and immunogenicity.
AU Brodin, Priscille; de Jonge, Marien I.; Majlessi, Laleh; Leclerc, Claude; Nilges, Michael; Cole, Stewart T.; Brosch, Roland [Reprint Author]
CS Inst Pasteur, Unite Genet Mol Bacterienne, 28 Rue Dr Roux, F-75724 Paris, France
rbrosch@pasteur.fr
SO Journal of Biological Chemistry, (OCT 7 2005) Vol. 280, No. 40, pp. 33953-33959.
CODEN: JBCHA3. ISSN: 0021-9258.
DT Article
LA English
ED Entered STN: 14 Dec 2005
Last Updated on STN: 14 Dec 2005
AB Proteins of the 6-kDa early secreted antigenic target (ESAT-6) secretion system-1 of Mycobacterium tuberculosis are not only strongly involved in the anti-mycobacterial Th1-host immune response but are also key players

for virulence. In this study, protein engineering together with bioinformatic, immunological, and virulence analyses allowed us to pinpoint regions of the ESAT-6 molecule that are critical for its biological activity in M. tuberculosis. Mutation of the Trp-Xaa-Gly motif, conserved in a wide variety of ESAT-6-like proteins, abolished complex formation with the partner protein CFP-10, induction of specific T-cell responses, and virulence. Replacement of conserved Leu residues interfered with secretion, coiled-coil formation, and virulence, whereas certain mutations at the extreme C terminus did not affect secretion but caused attenuation, possibly because of altered ESAT-6 targeting or trafficking. In contrast, the mutation of several residues on the outer surface of the four-helical bundle structure of the ESAT-6 center dot CFP-10 complex showed much less effect. Construction of recombinant BCG expressing ESAT-6 with a C-terminal hexahistidine tag allowed us to co-purify ESAT-6 and CFP-10, experimentally confirming their strong interaction both in and outside of the mycobacterial cell. The strain induced potent, antigen-specific T-cell responses and intermediate in vivo growth in mice, suggesting that it remained immunogenic and biologically active despite the tag. Together with previous NMR data, the results of this study have allowed a biologically relevant model of the ESAT-6 center dot CFP-10 complex to be constructed that is critical for understanding the structure-function relationship in tuberculosis pathogenesis.

L6 ANSWER 5 OF 23 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 4
AN 2005:215081 BIOSIS
DN PREV200510005668
TI Influence of ESAT-6 secretion system 1 (RD1) of Mycobacterium tuberculosis on the interaction between mycobacteria and the host immune system.
AU Majlessi, Laleh [Reprint Author]; Brodin, Priscille; Brosch, Roland; Rojas, Marie-Jesus; Khun, Huot; Huerre, Michel; Cole, Stewart T.; Leclerc, Claude
CS Inst Pasteur, Unite Biol Regulat Immun, INSERM, Equipe 352, 25,Rue Dr Roux, F-75724 Paris 15, France
lmajless@pasteur.fr
SO Journal of Immunology, (MAR 15 2005) Vol. 174, No. 6, pp. 3570-3579. CODEN: JOIMA3. ISSN: 0022-1767.
DT Article
LA English
ED Entered STN: 10 Jun 2005
Last Updated on STN: 10 Jun 2005
AB The chromosomal locus encoding the early secreted antigenic target, 6 kDa (ESAT-6) secretion system I of Mycobacterium tuberculosis, also referred to as "region of difference I (RD1)," is absent from Mycobacterium bovis bacillus Calmette-Guerin (BCG). In this study, using low-dose aerosol infection in mice, we demonstrate that BCG complemented with RD1 (BCG::RD1) displays markedly increased virulence which albeit does not attain that of M. tuberculosis H37Rv. Nevertheless, phenotypic and functional analyses of immune cells at the site of infection show that the capacity of BCG::RD1 to initiate recruitment/activation of immune cells is compaiable to that of fully virulent H37Rv. Indeed, in contrast to the parental BCG, BCG::RDI mimics H37Rv and induces substantial influx of activated (CD44(high)CD45RB(-)CD62L(-)) or effector (CD45RB(-)CD27(-)) T cells and of activated CD11c(+)CD11b(high) cells to the lungs of aerosol-infected mice. For the first time, using in vivo analysis of transcriptome of inflammatory cytokines and chemokines of lung interstitial CD11c(+) cells, we show that in a low-dose aerosol infection model, BCG::RDI triggered an activation/inflammation program comparable to that induced by H37Rv while parental BCG, due to its overattenuation, did not initiate the activation program in lung interstitial CD11c(+) cells. Thus, products encoded by the ESAT-6 secretion system 1 of M. tuberculosis profoundly modify the interaction between mycobacteria and the host innate and adaptive immune system. These modifications can explain the

previously described improved protective capacity of BCG::RDI vaccine candidate against M. tuberculosis challenge. The Journal of Immunology, 2005, 174: 3570-3579.

L6 ANSWER 6 OF 23 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 5
AN 2005:430578 BIOSIS
DN PREV200510218487
TI Modulation of the host immune response by a transient intracellular stage of Mycobacterium ulcerans: the contribution of endogenous mycolactone toxin.
AU Coutanceau, Emmanuelle; Marsollier, Laurent; Brosch, Roland; Perret, Emmanuelle; Goossens, Pierre; Tanguy, Myriam; Cole, Stewart T.; Small, Pamela L. C.; Demangel, Caroline [Reprint Author]
CS Inst Pasteur, Unite Genet Mol Bacterienne, Paris, France demangel@pasteur.fr
SO Cellular Microbiology, (AUG 2005) Vol. 7, No. 8, pp. 1187-1196. ISSN: 1462-5814.
DT Article
LA English
ED Entered STN: 26 Oct 2005
Last Updated on STN: 26 Oct 2005
AB Mycobacterium ulcerans (Mu), the aetiological agent of Buruli ulcer, is an extracellular pathogen producing the macrolide toxin mycolactone. Using a mouse model of intradermal infection, we found that Mu was initially captured by phagocytes and transported to draining lymph nodes (DLN) within host cells. Similar to Buruli ulcers in humans, the infection site eventually became ulcerated with tissue necrosis and extracellular bacteria, at later stages. In contrast to Mycobacterium bovis BCG (BCG), Mu did not disseminate to the spleen. However, mice infected with Mu or BCG developed comparable primary cellular responses to mycobacterial antigens in DLN and spleen. The role of mycolactone in this sequence of events was examined with a mycolactone-deficient (mup045) mutant of Mu. Mup045 bacilli were better internalized than wild-type (wt) bacteria by mouse phagocytes in vitro. Moreover, infection with wt but not mup045 Mu led to inhibition of TNF-alpha expression, upregulation of MIP-2 chemokine, and host cell death within 1 day. Our results suggest that mycolactone expression during the intracellular life of Mu may contribute to immune evasion by inhibiting phagocytosis, provoking apoptosis of antigen presenting cells and altering the establishment of an appropriate inflammatory reaction.

L6 ANSWER 7 OF 23 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 6
AN 2005:1076707 CAPLUS
DN 145:44411
TI Tuberculosis: From genome to vaccine
AU de Jonge, Marien I.; Brosch, Roland; Brodin, Priscille; Demangel, Caroline; Cole, Stewart T.
CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, Paris, 75724, Fr.
SO Expert Review of Vaccines (2005), 4(4), 541-551
CODEN: ERVXAX; ISSN: 1476-0584
PB Future Drugs Ltd.
DT Journal; General Review
LA English
AB A review. The availability of mycobacterial genome sequences has paved the way to identifying potential tuberculosis vaccine candidates in order to replace the currently used bacillus Calmette-Guerin (BCG) vaccines that show variable protective efficacy in adults. Genomics provides the basis for bioinformatic, transcriptomic and proteomic anal., increases screening efficiency and enables valuable information concerning the biol. and virulence of the mycobacterial species to be extracted by comparative genomics. Although in silico results must be confirmed in vitro and in vivo, bioinformatic anal. of the genomes is highlighting

candidates for testing. For designing subunit vaccines, attenuated or improved recombinant whole-cell live vaccines, information from the genomes of the human host and pathogenic mycobacterial species is of great help.

RE.CNT 111 THERE ARE 111 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 8 OF 23 CAPLUS COPYRIGHT 2007 ACS on STN
AN 2004:1050153 CAPLUS
DN 142:332541
TI Comparative genomics and evolution of Mycobacterium bovis BCG
AU Brosch, Roland; Behr, Marcel A.
CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, Paris, 75724, Fr.
SO Tuberculosis and the Tubercle Bacillus (2005), 155-164. Editor(s): Cole, Stewart T. Publisher: American Society for Microbiology, Washington, D. C. CODEN: 69GFRV; ISBN: 1-55581-295-3
DT Conference; General Review
LA English
AB A review. BCG, the live attenuated strain of Mycobacterium bovis, is one of the world's most widely used vaccines and the only anti-tuberculosis vaccine that is still used on a large scale. Mol. and immunol. characterization of the presently used BCG vaccine strains continues to be an important research topic, not only to elucidate the genetic basis for the attenuation of BCG but also to learn more about the genetic background of diverse BCG substrains that may be involved in the variable protective efficacy. Recent advances in the field of mycobacterial genetics, genomics, comparative genomics, and related techniques have provided an enormous amount of new information. Such new information is discussed.

RE.CNT 53 THERE ARE 53 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 9 OF 23 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 7
AN 2004:438631 BIOSIS
DN PREV200400437455
TI Cell envelope protein PPE68 contributes to Mycobacterium tuberculosis RDI immunogenicity independently of a 10-kilodalton culture filtrate protein and ESAT-6.
AU Demangel, Caroline [Reprint Author]; Brodin, Priscille; Cockle, Paul J.; Brosch, Roland; Majlessi, Laleh; Leclerc, Claude; Cole, Stewart T.
CS Unite Genet Mol Bacterienne, Inst Pasteur, 28 Rue Dr Roux, F-75724, Paris, 15, France
demangel@pasteur.fr
SO Infection and Immunity, (April 2004) Vol. 72, No. 4, pp. 2170-2176. print. ISSN: 0019-9567 (ISSN print).
DT Article
LA English
ED Entered STN: 17 Nov 2004
Last Updated on STN: 17 Nov 2004
AB The protective efficacy of Mycobacterium bovis BCG can be markedly augmented by stable integration of Mycobacterium tuberculosis genomic region RD1. BCG complemented with RD1 (BCG ::RDI) encodes nine additional proteins. Among them, 10-kDa culture filtrate protein (CFP-10) and ESAT-6 (6-kDa early secreted antigenic target) are low-molecular-weight proteins that induce potent Th1 responses. Using pools of synthetic peptides, we have examined the potential immunogenicity of four other RD1 products (PE35, PPE68, Rv3878, and Rv3879c). PPE68, the protein encoded by rv3873, was the only one to elicit gamma interferon (IFN-gamma)-producing cells in C57BL/6 mice infected with M. tuberculosis. Anti-PPE68 T cells were predominantly raised against an epitope mapped in the N-terminal end of the protein. Importantly, inactivation of rv3873 in BCG::RD1 did not modify

CFP-10 and ESAT-6 secretion. Moreover, the generation of IFN-gamma responses to these antigens following immunization with BCG::RD1 was independent of PPE68 expression. Taken together, these results show that PPE68 is an immunogenic product of the RD1 region, which does not interfere with the secretion and immunogenicity of CFP-10 and ESAT-6.

L6 ANSWER 10 OF 23 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 8
AN 2004:164676 BIOSIS
DN PREV200400168448
TI Macro-array and bioinformatic analyses reveal mycobacterial 'core' genes, variation in the ESAT-6 gene family and new phylogenetic markers for the Mycobacterium tuberculosis complex.
AU Marmiesse, Magali; Brodin, Priscille; Buchrieser, Carmen; Gutierrez, Christina; Simoes, Nathalie; Vincent, Veronique; Glaser, Philippe; Cole, Stewart T.; Brosch, Roland [Reprint Author]
CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, 25-28 Rue du Docteur Roux, 75724, Paris Cedex 15, France
rbrosch@pasteur.fr
SO Microbiology (Reading), (February 2004) Vol. 150, No. 2, pp. 483-496. print.
ISSN: 1350-0872 (ISSN print).
DT Article
LA English
ED Entered STN: 24 Mar 2004
Last Updated on STN: 24 Mar 2004
AB To better understand the biology and the virulence determinants of the two major mycobacterial human pathogens Mycobacterium tuberculosis and Mycobacterium leprae, their genome sequences have been determined recently. In silico comparisons revealed that among the 1439 genes common to both M. tuberculosis and M. leprae, 219 genes code for proteins that show no similarity with proteins from other organisms. Therefore, the latter 'core' genes could be specific for mycobacteria or even for the intracellular mycobacterial pathogens. To obtain more information as to whether these genes really were mycobacteria-specific, they were included in a focused macro-array, which also contained genes from previously defined regions of difference (RD) known to be absent from Mycobacterium bovis BCG relative to M. tuberculosis. Hybridization of DNA from 40 strains of the M. tuberculosis complex and in silico comparison of these genes with the near-complete genome sequences from Mycobacterium avium, Mycobacterium marinum and Mycobacterium smegmatis were undertaken to answer this question. The results showed that among the 219 conserved genes, very few were not present in all the strains tested. Some of these missing genes code for proteins of the ESAT-6 family, a group of highly immunogenic small proteins whose presence and number is variable among the genomically highly conserved members of the M. tuberculosis complex. Indeed, the results suggest that, with few exceptions, the 'core' genes conserved among M. tuberculosis H37Rv and M. leprae are also highly conserved among other mycobacterial strains, which makes them interesting potential targets for developing new specific anti-mycobacterial drugs. In contrast, the genes from RD regions showed great variability among certain members of the M. tuberculosis complex, and some new specific deletions in Mycobacterium canettii, Mycobacterium microti and seal isolates were identified and further characterized during this study. Together with the distribution of a particular 6 or 7 bp micro-deletion in the gene encoding the polyketide synthase pks15/1, these results confirm and further extend the revised phylogenetic model for the M. tuberculosis complex recently presented.

L6 ANSWER 11 OF 23 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 9
AN 2004:326990 BIOSIS
DN PREV200400328635
TI Enhanced protection against tuberculosis by vaccination with recombinant

Mycobacterium microti vaccine that induces T cell immunity against region of difference 1 antigens.

AU Brodin, Priscille; Majlessi, Laleh; Brosch, Roland; Smith, Debbie; Bancroft, Gregory; Clark, Simon; Williams, Ann; Leclerc, Claude; Cole, Stewart T. [Reprint Author]

CS Unite Genet Mol Bacterienne, Inst Pasteur, 28 Rue Docteur Roux, F-75724, Paris, 15, France
stcole@pasteur.fr

SO Journal of Infectious Diseases, (July 1 2004) Vol. 190, No. 1, pp. 115-122. print.
CODEN: JIDIAQ. ISSN: 0022-1899.

DT Article

LA English

ED Entered STN: 29 Jul 2004
Last Updated on STN: 29 Jul 2004

AB Mycobacterium microti, the vole bacillus, which was used as a live vaccine against tuberculosis until the 1970s, confers the same protection in humans as does Mycobacterium bovis bacille Calmette-Guerin (BCG). However, because the efficacy of the BCG vaccine varies considerably, we have tried to develop a better vaccine by reintroducing into M. microti the complete region of difference 1 (RD1), which is required for secretion of the potent T cell antigens early secreted antigen target (ESAT)-6 and culture filtrate protein (CFP)-10. The resultant recombinant strain, M. microti OV254::RD1-2F9, induced specific ESAT-6 and CFP-10 immune responses in mice with CD8+ T lymphocytes that had strong expression of the CD44hi activation marker. This vaccine also displayed better efficacy against disseminated disease in the mouse and the guinea pig models of tuberculosis than was seen in animals vaccinated with M. microti alone or with BCG. The M. microti OV254::RD1-2F9 vaccine was less virulent and persistent in mice and than was BCG ::RD1-2F9 may represent a safer alternative to BCG::RD1-2F9.

L6 ANSWER 12 OF 23 CAPLUS COPYRIGHT 2007 ACS on STN

AN 2003:791413 CAPLUS

DN 139:304478

TI Identification of virulence associated regions RD1 and RD5 enabling the development of improved vaccines of M. bovis BCG and M. microti

IN Cole, Stewart; Pym, Alexander S.; Brosch, Roland; Brodin, Priscille; Majlessi, Laleh; Leclerc, Claude

PA Institut Pasteur, Fr.

SO Eur. Pat. Appl., 58 pp.
CODEN: EPXXDW

DT Patent

LA English

FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI EP 1350839	A1	20031008	EP 2002-290864	20020405
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
CA 2481318	A1	20031016	CA 2003-2481318	20030401
WO 2003085098	A2	20031016	WO 2003-IB1789	20030401
WO 2003085098	A3	20040129		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR,				

BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

	AU 2003223039	A1	20031020	AU 2003-223039	20030401
	EP 1492867	A2	20050105	EP 2003-719008	20030401
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
	US 2005220811	A1	20051006	US 2004-510021	20041001
PRAI	EP 2002-290864	A	20020405		
	WO 2003-IB1789	W	20030401		

AB The present invention relates to a strain of Mycobacterium bovis BCG or Mycobacterium microti, wherein said strain has integrated part or all of the RD1 region responsible for enhanced immunogenicity of the tubercle bacilli, especially the ESAT-6 and CFP-10 genes. These strains will be referred as the M. bovis BCG::RD1 or M. microti::RD1 strains and are useful as a new improved vaccine for preventing tuberculosis and as a therapeutical product enhancing the stimulation of the immune system for the treatment of bladder cancer.

RE.CNT 11 THERE ARE 11 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 13 OF 23 CAPLUS COPYRIGHT 2007 ACS on STN
AN 2003:675581 CAPLUS
DN 139:208831
TI Species-specific deletions in Mycobacterium genomes and their use in diagnosis of tuberculosis and in vaccines
IN Cole, Stewart; Brosch, Roland; Gordon, Stephen; Eiglmeier, Karin; Garnier, Thierry
PA Institut Pasteur, Fr.
SO Eur. Pat. Appl., 73 pp.
CODEN: EPXXDW
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 1338657	A1	20030827	EP 2002-290458	20020225
	EP 1338657	B1	20070418		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
	AT 360097	T	20070515	AT 2002-290458	20020225
	CA 2477195	A1	20030828	CA 2003-2477195	20030225
	WO 2003070981	A2	20030828	WO 2003-IB986	20030225
	WO 2003070981	A3	20031204		
	WO 2003070981	A8	20041007		
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
	AU 2003208539	A1	20030909	AU 2003-208539	20030225
	EP 1478777	A2	20041124	EP 2003-706832	20030225
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
	JP 2005518203	T	20050623	JP 2003-569872	20030225
	US 2006127897	A1	20060615	US 2006-505405	20060113
PRAI	EP 2002-290458	A	20020225		
	WO 2003-IB986	W	20030225		

AB Genomic deletions in different species of Mycobacterium which make it possible in particular to distinguish an infection resulting from Mycobacterium tuberculosis from an infection resulting from M. africanum,

M. canettii, M. microti, M. bovis and Mycobacterium BCG are identified. These markers can be used in cases where others markers, such as 16S rRNA genes or IS6110, or mutation in the katG gene are absent or uninformative. The subject of the present invention is also a method for detecting the sequences in question by the products of expression of these sequences and the kits for carrying out these methods. Finally, the subject of the present invention is novel vaccines. Three markers are described in detail. These are TbD1, which is deleted from the M. tuberculosis genome; RD4 deleted from M. bovis and Mycobacterium BCG, and RD9 deleted from M. bovis, Mycobacterium BCG, and M. africanum. Primers for the detection of these sequences are reported.

RE.CNT 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 14 OF 23 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 10

AN 2003:249243 BIOSIS

DN PREV200300249243

TI Recombinant BCG exporting ESAT-6 confers enhanced protection against tuberculosis.

AU Pym, Alexander S.; Brodin, Priscille; Majlessi, Laleh; Brosch, Roland; Demangel, Caroline; Williams, Ann; Griffiths, Karen E.; Marchal, Gilles; Leclerc, Claude; Cole, Stewart T. [Reprint Author]

CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, Paris, France
stcole@pasteur.fr

SO Nature Medicine, (May 2003) Vol. 9, No. 5, pp. 533-539. print.
ISSN: 1078-8956 (ISSN print).

DT Article

LA English

ED Entered STN: 28 May 2003
Last Updated on STN: 28 May 2003

AB The live tuberculosis vaccines Mycobacterium bovis BCG (bacille Calmette-Guerin) and Mycobacterium microti both lack the potent, secreted T-cell antigens ESAT-6 (6-kDa early secretory antigenic target) and CFP-10 (10-kDa culture filtrate protein). This is a result of independent deletions in the region of deletion-1 (RD1) locus, which is intact in virulent members of the Mycobacterium tuberculosis complex. To increase their immunogenicity and protective capacity, we complemented both vaccines with different constructs containing the esxA and esxB genes, which encode ESAT-6 and CFP-10 respectively, as well as a variable number of flanking genes. Only reintroduction of the complete locus, comprising at least 11 genes, led to full secretion of the antigens and resulted in specific ESAT-6-dependent immune responses; this suggests that the flanking genes encode a secretory apparatus. Mice and guinea pigs vaccinated with the recombinant strain BCG::RD1-2F9 were better protected against challenge with M. tuberculosis, showing less severe pathology and reduced dissemination of the pathogen, as compared with control animals immunized with BCG alone.

L6 ANSWER 15 OF 23 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 11

AN 2002:559623 BIOSIS

DN PREV200200559623

TI Bacterial artificial chromosome-based comparative genomic analysis identifies Mycobacterium microti as a natural ESAT-6 deletion mutant.

AU Brodin, Priscille; Eiglmeier, Karin; Marmiesse, Magali; Billault, Alain; Garnier, Thierry; Niemann, Stefan; Cole, Stewart T.; Brosch, Roland [Reprint author]

CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, 28 Rue du Docteur Roux, 75724, Paris Cedex 15, France
rbrosch@pasteur.fr

SO Infection and Immunity, (October, 2002) Vol. 70, No. 10, pp. 5568-5578. print.
CODEN: INFIBR. ISSN: 0019-9567.
DT Article
LA English
ED Entered STN: 30 Oct 2002
Last Updated on STN: 30 Oct 2002
AB Mycobacterium microti is a member of the Mycobacterium tuberculosis complex that causes tuberculosis in voles. Most strains of M. microti are harmless for humans, and some have been successfully used as live tuberculosis vaccines. In an attempt to identify putative virulence factors of the tubercle bacilli, genes that are absent from the avirulent M. microti but present in human pathogen M. tuberculosis or Mycobacterium bovis were searched for. A minimal set of 50 bacterial artificial chromosome (BAC) clones that covers almost all of the genome of M. microti OV254 was constructed, and individual BACs were compared to the corresponding BACs from M. bovis AF2122/97 and M. tuberculosis H37Rv. Comparison of pulsed-field gel-separated DNA digests of BAC clones led to the identification of 10 regions of difference (RD) between M. microti OV254 and M. tuberculosis. A 14-kb chromosomal region (RD1mic) that partly overlaps the RD1 deletion in the BCG vaccine strain was missing from the genomes of all nine tested M. microti strains. This region covers 13 genes, Rv3864 to Rv3876, in M. tuberculosis, including those encoding the potent ESAT-6 and CFP-10 antigens. In contrast, RD5mic, a region that contains three phospholipase C genes (plcA to -C), was missing from only the vole isolates and was present in M. microti strains isolated from humans . Apart from RD1mic and RD5mic other M. microti-specific deleted regions have been identified (MiD1 to MiD3). Deletion of MiD1 has removed parts of the direct repeat region in M. microti and thus contributes to the characteristic spoligotype of M. microti strains.

L6 ANSWER 16 OF 23 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 12
AN 2002:580808 CAPLUS
DN 138:67299
TI Rapid and simple approach for identification of Mycobacterium tuberculosis complex isolates by PCR-based genomic deletion analysis
AU Parsons, Linda M.; Brosch, Roland; Cole, Stewart T.; Somoskovi, Akos; Loder, Arthur; Bretzel, Gisela; van Soolingen, Dick; Hale, Yvonne M.; Salfinger, Max
CS Wadsworth Center, New York State Department of Health, Albany, NY, USA
SO Journal of Clinical Microbiology (2002), 40(7), 2339-2345
CODEN: JCMIDW; ISSN: 0095-1137
PB American Society for Microbiology
DT Journal
LA English
AB Although the virulences and host ranges differ among members of the Mycobacterium tuberculosis complex (TBC; M. tuberculosis, M. africanum, M. canettii, M. microti, M. bovis, and M. bovis BCG), com. available mol. assays cannot differentiate these organisms because of the genetic identities of their 16S rRNA gene sequences. Comparative genomic analyses with the complete DNA sequence of M. tuberculosis H37Rv has provided information on regions of difference (RD 1 to RD 16) deleted in members of the TBC other than M. tuberculosis. To determine whether deletion anal. could accurately differentiate members of TBC, we used PCR to assess the presence or absence of specific regions of the genome in 88 well-characterized isolates of M. tuberculosis, M. africanum, M. microti, M. bovis, and M. bovis BCG. The identifications obtained by use of the specific deletion profiles correlated 100% with the original identifications for all TBC members except M. africanum, but further characterization resulted in profiles specific for all members. Although six RD regions were used in the analyses with the original 88 isolates, it was found that the use of RD 1,

RD 9, and RD 10 was sufficient for initial screenings, followed by the use of RD 3, RD 5, and RD 11 if the results for any of the first three regions were neg. When 605 sequential clin. isolates were screened, 578 (96%) were identified as M. tuberculosis, 6 (1%) were identified as M. africanum, 8 (1%) were identified as M. bovis, and 13 (2%) were identified as M. bovis BCG. Since PCR-based assays can be implemented in most clin. mycobacteriol. labs., this approach provides a rapid and simple means for the differentiation of members of TBC, especially M. bovis and M. tuberculosis, when it is important to distinguish between zoonotic sources (i.e., cattle and unpasteurized dairy products) and human sources of tuberculosis disease.

RE.CNT 41 THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 17 OF 23 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 13

AN 2002:614464 BIOSIS

DN PREV200200614464

TI Loss of RD1 contributed to the attenuation of the live tuberculosis vaccines Mycobacterium bovis BCG and Mycobacterium microti.

AU Pym, Alexander S.; Brodin, Priscille; Brosch, Roland; Huerre, Michel; Cole, Stewart T. [Reprint author]

CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, 28 Rue du Docteur Roux, 75724, Paris Cedex 15, France
stcole@pasteur.fr

SO Molecular Microbiology, (November, 2002) Vol. 46, No. 3, pp. 709-717. print.
CODEN: MOMIEE. ISSN: 0950-382X.

DT Article

LA English

ED Entered STN: 4 Dec 2002
Last Updated on STN: 4 Dec 2002

AB Although large human populations have been safely immunized against tuberculosis with two live vaccines, Mycobacterium bovis BCG or Mycobacterium microti, the vole bacillus, the molecular basis for the avirulence of these vaccine strains remains unknown. Comparative genomics has identified a series of chromosomal deletions common to both virulent and avirulent species but only a single locus, RD1, that has been deleted from M. bovis BCG and M. microti. Restoration of RD1, by gene knock-in, resulted in a marked change in colonial morphology towards that of virulent tubercle bacilli. Three RD1-encoded proteins were localized in the cell wall, and two of them, the immunodominant T-cell antigens ESAT-6 and CFP-10, were also found in culture supernatants. The BCG::RD1 and M. microti ::RD1 knock-ins grew more vigorously than controls in immunodeficient mice, inducing extensive splenomegaly and granuloma formation. Increased persistence and partial reversal of attenuation were observed when immunocompetent mice were infected with the BCG::RD1 knock-in, whereas BCG controls were cleared. Knocking-in five other RD loci did not affect the virulence of BCG. This study describes a genetic lesion that contributes to safety and opens new avenues for vaccine development.

L6 ANSWER 18 OF 23 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 14

AN 2001:465041 BIOSIS

DN PREV200100465041

TI The evolution of mycobacterial pathogenicity: Clues from comparative genomics.

AU Brosch, Roland [Reprint author]; Pym, Alexander S. [Reprint author]; Gordon, Stephen V.; Cole, Stewart T. [Reprint author]

CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, 28 Rue du Dr Roux, 75724, Paris Cedex 15, France

stcole@pasteur.fr
SO Trends in Microbiology, (September, 2001) Vol. 9, No. 9, pp. 452-458. print.
ISSN: 0966-842X.
DT Article
General Review; (Literature Review)
LA English
ED Entered STN: 3 Oct 2001
Last Updated on STN: 23 Feb 2002
AB Comparative genomics, and related technologies, are helping to unravel the molecular basis of the pathogenesis, host range, evolution and phenotypic differences of the slow-growing mycobacteria. In the highly conserved Mycobacterium tuberculosis complex, where single-nucleotide polymorphisms are rare, insertion and deletion events (InDels) are the principal source of genome plasticity. InDels result from recombinational or insertion sequence (IS)-mediated events, expansion of repetitive DNA sequences, or replication errors based on repetitive motifs that remove blocks of genes or contract coding sequences. Comparative genomic analyses also suggest that loss of genes is part of the ongoing evolution of the slow-growing mycobacterial pathogens and might also explain how the vaccine strain BCG became attenuated.

L6 ANSWER 19 OF 23 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 15
AN 2001:49988 BIOSIS
DN PREV200100049988
TI Tools for the population genomics of the tubercle bacilli.
AU Pym, Alexander S.; Brosch, Roland [Reprint author]
CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, 75724, Paris Cedex 15, France
rbrosch@pasteur.fr
SO Genome Research, (December, 2000) Vol. 10, No. 12, pp. 1837-1839. print.
ISSN: 1088-9051.
DT Article
LA English
ED Entered STN: 24 Jan 2001
Last Updated on STN: 12 Feb 2002

L6 ANSWER 20 OF 23 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
AN 2000:430291 BIOSIS
DN PREV200000430291
TI Comparative genomics of the mycobacteria.
AU Brosch, Roland [Reprint author]; Gordon, Stephen V.; Pym, Alexander; Eiglmeier, Karin; Garnier, Thierry; Cole, Stewart T.
CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, 28 rue du Docteur Roux, 75724, Paris Cedex, 15, France
SO IJMM International Journal of Medical Microbiology, (May, 2000) Vol. 290, No. 2, pp. 143-152. print.
ISSN: 1438-4221.
DT Article
General Review; (Literature Review)
LA English
ED Entered STN: 11 Oct 2000
Last Updated on STN: 10 Jan 2002
AB The genus mycobacteria includes two important human pathogens Mycobacterium tuberculosis and Mycobacterium lepra. The former is reputed to have the highest annual global mortality of all pathogens. Their slow growth, virulence for humans and particular physiology makes these organisms extremely difficult to work with. However the rapid development of mycobacterial genomics following the completion of the Mycobacterium tuberculosis genome sequence provides the basis for a powerful new approach for the understanding of these organisms. Five further genome sequencing projects of closely related mycobacterial species with

differing host range, virulence for humans and physiology are underway. A comparative genomic analysis of these species has the potential to define the genetic basis of these phenotypes which will be invaluable for the development of urgently needed new vaccines and drugs. This minireview summarises the different techniques that have been employed to compare these genomes and gives an overview of the wealth of data that has already been generated by mycobacterial comparative genomics.

L6 ANSWER 21 OF 23 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 16
AN 2000:418561 BIOSIS
DN PREV200000418561
TI Comparative genomics uncovers large tandem chromosomal duplications in Mycobacterium bovis BCG pasteur.
AU Brosch, Roland; Gordon, Stephen V.; Buchrieser, Carmen; Pym, Alexander S.; Garnier, Thierry; Cole, Stewart T. [Reprint author]
CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, 28 Rue du Dr Roux, 75724, Paris Cedex, 15, France
SO Yeast, (30 June, 2000) Vol. 17, No. 2, pp. 111-123. print.
CODEN: YESTE3. ISSN: 0749-503X.
DT Article
LA English
ED Entered STN: 4 Oct 2000
Last Updated on STN: 8 Jan 2002
AB On direct comparison of minimal sets of ordered clones from bacterial artificial chromosome (BAC) libraries representing the complete genomes of Mycobacterium tuberculosis H37Rv and the vaccine strain, Mycobacterium bovis BCG Pasteur, two major rearrangements were identified in the genome of M. bovis BCG Pasteur. These were shown to correspond to two tandem duplications, DU1 and DU2, of 29 668 bp and 36 161 bp, respectively. While DU1 resulted from a single duplication event, DU2 apparently arose from duplication of a 100 kb genomic segment that subsequently incurred an internal deletion of 64 kb. Several lines of evidence suggest that DU2 may continue to expand, since two copies were detected in a subpopulation of BCG Pasteur cells. BCG strains harbouring DU1 and DU2 are diploid for at least 58 genes and contain two copies of oriC, the chromosomal origin of replication. These findings indicate that these genomic regions of the BCG genome are still dynamic. Although the role of DU1 and DU2 in the attenuation and/or altered immunogenicity of BCG is yet unknown, knowledge of their existence will facilitate quality control of BCG vaccine lots and may help in monitoring the efficacy of the world's most widely used vaccine.

L6 ANSWER 22 OF 23 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 17
AN 1999:299929 BIOSIS
DN PREV199900299929
TI Identification of variable regions in the genomes of tubercle bacilli using bacterial artificial chromosome arrays.
AU Gordon, Stephen V.; Brosch, Roland; Billault, Alain; Garnier, Thierry; Eiglmeier, Karin; Cole, Stewart T. [Reprint author]
CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, 28 Rue du Dr Roux, 75724, Paris, Cedex 15, France
SO Molecular Microbiology, (May, 1999) Vol. 32, No. 3, pp. 643-655. print.
CODEN: MOMIEE. ISSN: 0950-382X.
DT Article
LA English
ED Entered STN: 12 Aug 1999
Last Updated on STN: 12 Aug 1999
AB Whole-genome comparisons of the tubercle bacilli were undertaken using ordered bacterial artificial chromosome (BAC) libraries of Mycobacterium tuberculosis and the vaccine strain, Mycobacterium bovis BCG -Pasteur, together with the complete genome sequence of M. tuberculosis

H37Rv. Restriction-digested BAC arrays of M. tuberculosis H37Rv were used in hybridization experiments with radiolabelled M. bovis BCG genomic DNA to reveal the presence of 10 deletions (RD1-RD10) relative to M. tuberculosis. Seven of these regions, RD4-RD10, were also found to be deleted from M. bovis, with the three M. bovis BCG-specific deletions being identical to the RD1-RD3 loci described previously. The distribution of RD4-RD10 in Mycobacterium africanum resembles that of M. tuberculosis more closely than that of M. bovis, whereas an intermediate arrangement was found in Mycobacterium microti, suggesting that the corresponding genes may affect host range and virulence of the various tubercle bacilli. Among the known products encoded by these loci are a copy of the proposed mycobacterial invasin Mce, three phospholipases, several PE, PPE and ESAT-6 proteins, epoxide hydrolase and an insertion sequence. In a complementary approach, direct comparison of BACs uncovered a third class of deletions consisting of two M. tuberculosis H37Rv loci, RvD1 and RvD2, deleted from the genome relative to M. bovis BCG and M. bovis. These deletions affect a further seven genes, including a fourth phospholipase, plcD. In summary, the insertions and deletions described here have important implications for our understanding of the evolution of the tubercle complex.

L6 ANSWER 23 OF 23 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 18

AN 1998:256186 BIOSIS

DN PREV199800256186

TI Use of a Mycobacterium tuberculosis H37Rv bacterial artificial chromosome library for genome mapping, sequencing, and comparative genomics.

AU Brosch, Roland; Gordon, Stephen V.; Billault, Alain; Garnier, Thierry; Eiglmeier, Karin; Soravito, Catherine; Barrell, Bart G.; Cole, Stewart T. [Reprint author]

CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, 28 Rue de Dr. Roux, 75724 Paris Cedex 15, France

SO Infection and Immunity, (May, 1998) Vol. 66, No. 5, pp. 2221-2229. print. CODEN: INFIBR. ISSN: 0019-9567.

DT Article

LA English

ED Entered STN: 9 Jun 1998
Last Updated on STN: 9 Jun 1998

AB The bacterial artificial chromosome (BAC) cloning system is capable of stably propagating large, complex DNA inserts in Escherichia coli. As part of the Mycobacterium tuberculosis H37Rv genome sequencing project, a BAC library was constructed in the pBeloBACII vector and used for genome mapping, confirmation of sequence assembly, and sequencing. The library contains about 5,000 BAC clones, with inserts ranging in size from 25 to 104 kb, representing theoretically a 70-fold coverage of the M. tuberculosis genome (4.4 Mb). A total of 840 sequences from the T7 and SP6 termini of 420 BACs were determined and compared to those of a partial genomic database. These sequences showed excellent correlation between the estimated sizes and positions of the BAC clones and the sizes and positions of previously sequenced cosmids and the resulting contigs. Many BAC clones represent linking clones between sequenced cosmids, allowing full coverage of the H37Rv chromosome, and they are now being shotgun sequenced in the framework of the H37Rv sequencing project. Also, no chimeric, deleted, or rearranged BAC clones were detected, which was of major importance for the correct mapping and assembly of the H37Rv sequence. The minimal overlapping set contains 68 unique BAC clones and spans the whole H37Rv chromosome with the exception of a single gap of apprx 150 kb. As a postgenomic application, the canonical BAC set was used in a comparative study to reveal chromosomal polymorphisms between M. tuberculosis, M. bovis, and M. bovis BCG Pasteur, and a novel 12.7-kb segment present in M. tuberculosis but absent from M. bovis and M. bovis BCG was characterized. This region contains a set of genes whose products show low similarity to proteins involved in polysaccharide biosynthesis. The H37Rv BAC library therefore provides us

with a powerful tool both for the generation and confirmation of sequence data as well as for comparative genomics and other postgenomic applications. It represents a major resource for present and future M. tuberculosis research projects.

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=> e brodin priscille/au
E1            2     BRODIN PERSSON G/AU
E2           38     BRODIN PETER/AU
E3           86 --> BRODIN PRISCILLE/AU
E4            1     BRODIN PRISCILLE MARIE MONIQUE/AU
E5           17     BRODIN R/AU
E6           22     BRODIN ROGER/AU
E7            8     BRODIN S/AU
E8           20     BRODIN S V/AU
E9            1     BRODIN SARTORIUS ALBANE/AU
E10           1     BRODIN STAFFAN/AU
E11          95     BRODIN T/AU
E12          16     BRODIN T N/AU

=> s e3-e4 and (bcg or microti)
L7           53 ("BRODIN PRISCILLE"/AU OR "BRODIN PRISCILLE MARIE MONIQUE"/AU)
                AND (BCG OR MICROTI)

=> dup rem l7
PROCESSING COMPLETED FOR L7
L8           18 DUP REM L7 (35 DUPLICATES REMOVED)

=> d bib ab 1-
YOU HAVE REQUESTED DATA FROM 18 ANSWERS - CONTINUE? Y/(N):y

L8   ANSWER 1 OF 18  CAPLUS  COPYRIGHT 2007 ACS on STN
AN   2007:41383  CAPLUS
DN   146:140994
TI   Modified ESAT-6 derived from Mycobacterium tuberculosis and Mycobacterium
     leprae as vaccines for inducing interferon γ response to ESAT-6
     and/or CFP-10 against infection
IN   Brosch, Roland; Brodin, Priscille; Cole, Stewart; Majlessi,
     Laleh; Leclerc, Claude
PA   Fr.
SO   U.S. Pat. Appl. Publ., 64pp.
     CODEN: USXXCO
DT   Patent
LA   English
FAN.CNT 1
     PATENT NO.          KIND   DATE        APPLICATION NO.        DATE
     --------------      ----   --------    --------------------   --------
PI   US 2007009547        A1    20070111    US 2006-455929         20060620
     WO 2007010413        A2    20070125    WO 2006-IB2884         20060622
     WO 2007010413        A3    20070830
          W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH,
              CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD,
              GE, GH, GM, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP,
              KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN,
              MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU,
              SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG,
              US, UZ, VC, VN, ZA, ZM, ZW
          RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE,
              IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ,
              CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH,
              GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY,
              KG, KZ, MD, RU, TJ, TM, AP, EA, EP, OA
PRAI US 2005-692561P      P     20050622
AB   A genetically modified strain of M. tuberculosis or Mycobacterium bovis
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BCG is provided, wherein the genetically modified strain comprises at least one modified sequence comprising SEQ ID NO: 1, SEQ ID NO: 2, or both, having at least one mutation at T2, Q4, F8, A14, L28, L29, W43, G45, Q55, Q56, N66, M83, V90, M93, or F94. In a preferred embodiment, the mutation is at least one of T2H, Q4L, F8I, A14R, L28A, L29S, W43R, G45T, Y51, Q55I, Q56A, N66I, N66A, M83I, V90R, M93T, or F94Q. Similarly, the genetically modified strain may also secrete ESAT-6 with a a histidine tag, tetra-cysteine tag or FLAG-tag, a GFP-fusion, or a short truncation at the C-terminal end of less than 20 amino acids.

L8 ANSWER 2 OF 18 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 1
AN 2006:351223 BIOSIS
DN PREV200600347024
TI High frequency of CD4(+) T cells specific for the TB10.4 protein correlates with protection against Mycobacterium tuberculosis infection.
AU Hervas-Stubbs, Sandra; Majlessi, Laleh; Simsova, Marcela; Morova, Jana; Rojas, Marie-Jesus; Nouze, Clemence; Brodin, Priscille; Sebo, Peter; Leclerc, Claude [Reprint Author]
CS Inst Pasteur, INSERM, E352, 25 Rue Docteur Roux, F-75724 Paris 15, France cleclerc@pasteur.fr
SO Infection and Immunity, (JUN 2006) Vol. 74, No. 6, pp. 3396-3407. CODEN: INFIBR. ISSN: 0019-9567.
DT Article
LA English
ED Entered STN: 12 Jul 2006
Last Updated on STN: 12 Jul 2006
AB TB10.4 is a newly identified antigen of Mycobacterium tuberculosis recognized by human and murine T cells upon mycobacterial infection. Here, we show that immunization with Mycobacterium bovis BCG induces a strong, genetically controlled, Th1 immune response against TB10.4 in mice. BALB/c and C57BL/6 strains behave as high and low responders to TB10.4 protein, respectively. The TB10.4:74-88 peptide was identified as an immunodominant CD4(+) T-cell epitope for H-2(d) mice. Since recent results, as well as the present study, have raised interest in TB10.4 as a subunit vaccine, we analyzed immune responses induced by this antigen delivered by a new vector, the adenylate cyclase (CyaA) of Bordetella pertussis. CyaA is able to target dendritic cells and to deliver CD4(+) or CD8(+) T-cell epitopes to the major histocompatibility complex class II/I molecule presentation pathways, triggering specific Th1 or cytotoxic T-lymphocyte (CTL) responses. Several CyaA harboring either the entire TB10.4 protein or various subfragments containing the TB10.4:20-28 CTL epitope were shown to induce TB10.4-specific Th1 CD4(+) and CD8(+) T-cell responses. However, none of the recombinant CyaA, injected in the absence of adjuvant, was able to induce protection against M. tuberculosis infection. In contrast, TB10.4 protein administered with a cocktail of strong adjuvants that triggered a strong Th1 CD4(+) T-cell response induced significant protection against M. tuberculosis challenge. These results confirm the potential value of the TB10.4 protein as a candidate vaccine and show that the presence of high frequencies of CD4(+) T cells specific to this strong immunogen correlates with protection against M. tuberculosis infection.

L8 ANSWER 3 OF 18 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 2
AN 2006:370142 BIOSIS
DN PREV200600369174
TI An increase in antimycobacterial Th1-cell responses by prime-boost protocols of immunization does not enhance protection against tuberculosis.
AU Majlessi, Laleh [Reprint Author]; Simsova, Marcela; Jarvis, Zdenka; Brodin, Priscille; Rojas, Marie-Jesus; Bauche, Cecile; Nouze, Clemence; Ladant, Daniel; Cole, Stewart T.; Sebo, Peter; Leclerc, Claude
CS Inst Pasteur, Unite Biol Regulat Immunitaries, INSERM, 25 Rue Dr Roux,E

352, F-75724 Paris 15, France
lmajless@pasteur.fr
SO Infection and Immunity, (APR 2006) Vol. 74, No. 4, pp. 2128-2137.
CODEN: INFIBR. ISSN: 0019-9567.
DT Article
LA English
ED Entered STN: 26 Jul 2006
Last Updated on STN: 26 Jul 2006
AB Bordetella pertussis adenylate cyclase (CyaA) toxoid is a powerful nonreplicative immunization vector targeting dendritic cells, which has already been used successfully in prophylactic and therapeutic vaccination in various preclinical animal models. Here, we investigated the potential of CyaA, harboring strong mycobacterial immunogens, i.e., the immunodominant regions of antigen 85A or the complete sequence of the 6-kDa early secreted antigenic target (ESAT-6) protein, to induce anti mycobacterial immunity. By generating T-cell hybridomas or by using T cells from mice infected with mycobacteria, we first demonstrated that the in vitro delivery of 85A or ESAT-6 to antigen-presenting cells by CyaA leads to processing and presentation, by major histocompatibility complex class II molecules, of the same epitopes as those displayed upon mycobacterial infection. Importantly, compared to the recombinant protein alone, the presentation of ESAT-6 in vitro was 100 times more efficient upon its delivery to antigen-presenting cells in fusion to CyaA. Immunization with CyaA-85A or CyaA-ESAT-6 in the absence of any adjuvant induced strong antigen-specific lymphoproliferative, interleukin-2 (IL-2) and gamma interferon (IFN-gamma) cytokine responses, in the absence of any IL-4 or IL-5 production. When used as boosters after priming with a BCG expressing ESAT-6, the CyaA-85A and CyaA-ESAT-6 proteins were able to strikingly increase the sensitivity and intensity of proliferative and Th1-polarized responses and notably the frequency of antigen-specific IFN-gamma-producing CD4(+) T cells. However, immunization with these CyaA constructs as subunit vaccines alone or as boosters did not allow induction or improvement of protection against Mycobacterium tuberculosis infection. These results question the broadly admitted correlation between the frequency of IFN-gamma-producing CD4(+) T cells and the level of protection against tuberculosis.

L8 ANSWER 4 OF 18 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 3
AN 2006:176683 BIOSIS
DN PREV200600166449
TI Dissection of ESAT-6 system 1 of Mycobacterium tuberculosis and impact on immunogenicity and virulence.
AU Brodin, Priscille; Majlessi, Laleh; Marsollier, Laurent; de Jonge, Marien I.; Bottai, Daria; Demangel, Caroline; Hinds, Jason; Neyrolles, Olivier; Butcher, Philip D.; Leclerc, Claude; Cole, Stewart T.; Brosch, Roland [Reprint Author]
CS Inst Pasteur, Unite Genet Mol Bacterienne, 25-28 Rue Docteur Roux, F-75724 Paris 15, France
rbrosch@pasteur.fr
SO Infection and Immunity, (JAN 2006) Vol. 74, No. 1, pp. 88-98.
CODEN: INFIBR. ISSN: 0019-9567.
DT Article
LA English
ED Entered STN: 9 Mar 2006
Last Updated on STN: 9 Mar 2006
AB The dedicated secretion system ESX-1 of Mycobacterium tuberculosis encoded by the extended RD1 region (extRD1) assures export of the ESAT-6 protein and its partner, the 10-kDa culture filtrate protein CFP-10, and is missing from the vaccine strains M. bovis BCG and M. microti. Here, we systematically investigated the involvement of each individual ESX-1 gene in the secretion of both antigens, specific immunogenicity, and virulence. ESX-1-complemented BCG and M. microti strains were more efficiently engulfed by

bone-marrow-derived macrophages than controls, and this may account for the enhanced in vivo growth of ESX-1-carrying strains. Inactivation of gene pe35 (Rv3872) impaired expression of CFP-10 and ESAT-6, suggesting a role in regulation. Genes Rv3868, Rv3869, Rv3870, Rv3871, and Rv3877 encoding an ATP-dependent chaperone and translocon were essential for secretion of ESAT-6 and CFP-10 in contrast to ppe68 Rv3873 and Rv3876, whose inactivation did not impair secretion of ESAT-6. A strict correlation was found between ESAT-6 export and the generation of ESAT-6 specific T-cell responses in mice. Furthermore, ESAT-6 secretion and specific immunogenicity were almost always correlated with enhanced virulence in the SCID mouse model. Only loss of Rv3865 and part of Rv3866 did not affect ESAT-6 secretion or immunogenicity but led to attenuation. This suggests that Rv3865/66 represent a new virulence factor that is independent from ESAT-6 secretion. The present study has allowed us to identify new aspects of the extRD1 region of M. tuberculosis and to explore its role in the pathogenesis of tuberculosis.

L8 ANSWER 5 OF 18 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 4
AN 2006:7638 BIOSIS
DN PREV200600007409
TI Functional analysis of early secreted antigenic target-6, the dominant T-cell antigen of Mycobacterium tuberculosis, reveals key residues involved in secretion, complex formation, virulence, and immunogenicity.
AU Brodin, Priscille; de Jonge, Marien I.; Majlessi, Laleh; Leclerc, Claude; Nilges, Michael; Cole, Stewart T.; Brosch, Roland [Reprint Author]
CS Inst Pasteur, Unite Genet Mol Bacterienne, 28 Rue Dr Roux, F-75724 Paris, France
rbrosch@pasteur.fr
SO Journal of Biological Chemistry, (OCT 7 2005) Vol. 280, No. 40, pp. 33953-33959.
CODEN: JBCHA3. ISSN: 0021-9258.
DT Article
LA English
ED Entered STN: 14 Dec 2005
Last Updated on STN: 14 Dec 2005
AB Proteins of the 6-kDa early secreted antigenic target (ESAT-6) secretion system-1 of Mycobacterium tuberculosis are not only strongly involved in the anti-mycobacterial Th1-host immune response but are also key players for virulence. In this study, protein engineering together with bioinformatic, immunological, and virulence analyses allowed us to pinpoint regions of the ESAT-6 molecule that are critical for its biological activity in M. tuberculosis. Mutation of the Trp-Xaa-Gly motif, conserved in a wide variety of ESAT-6-like proteins, abolished complex formation with the partner protein CFP-10, induction of specific T-cell responses, and virulence. Replacement of conserved Leu residues interfered with secretion, coiled-coil formation, and virulence, whereas certain mutations at the extreme C terminus did not affect secretion but caused attenuation, possibly because of altered ESAT-6 targeting or trafficking. In contrast, the mutation of several residues on the outer surface of the four-helical bundle structure of the ESAT-6 center dot CFP-10 complex showed much less effect. Construction of recombinant BCG expressing ESAT-6 with a C-terminal hexahistidine tag allowed us to co-purify ESAT-6 and CFP-10, experimentally confirming their strong interaction both in and outside of the mycobacterial cell. The strain induced potent, antigen-specific T-cell responses and intermediate in vivo growth in mice, suggesting that it remained immunogenic and biologically active despite the tag. Together with previous NMR data, the results of this study have allowed a biologically relevant model of the ESAT-6 center dot CFP-10 complex to be constructed that is critical for understanding the structure-function relationship in tuberculosis pathogenesis.

L8 ANSWER 6 OF 18 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN

DUPLICATE 5
AN 2005:215081 BIOSIS
DN PREV200510005668
TI Influence of ESAT-6 secretion system 1 (RD1) of Mycobacterium tuberculosis on the interaction between mycobacteria and the host immune system.
AU Majlessi, Laleh [Reprint Author]; Brodin, Priscille; Brosch, Roland; Rojas, Marie-Jesus; Khun, Huot; Huerre, Michel; Cole, Stewart T.; Leclerc, Claude
CS Inst Pasteur, Unite Biol Regulat Immun, INSERM, Equipe 352, 25,Rue Dr Roux, F-75724 Paris 15, France
lmajless@pasteur.fr
SO Journal of Immunology, (MAR 15 2005) Vol. 174, No. 6, pp. 3570-3579. CODEN: JOIMA3. ISSN: 0022-1767.
DT Article
LA English
ED Entered STN: 10 Jun 2005
Last Updated on STN: 10 Jun 2005
AB The chromosomal locus encoding the early secreted antigenic target, 6 kDa (ESAT-6) secretion system I of Mycobacterium tuberculosis, also referred to as "region of difference I (RD1)," is absent from Mycobacterium bovis bacillus Calmette-Guerin (BCG). In this study, using low-dose aerosol infection in mice, we demonstrate that BCG complemented with RD1 (BCG::RD1) displays markedly increased virulence which albeit does not attain that of M. tuberculosis H37Rv. Nevertheless, phenotypic and functional analyses of immune cells at the site of infection show that the capacity of BCG::RD1 to initiate recruitment/activation of immune cells is compaiable to that of fully virulent H37Rv. Indeed, in contrast to the parental BCG, BCG::RDI mimics H37Rv and induces substantial influx of activated (CD44(high)CD45RB(-)CD62L(-)) or effector (CD45RB(-)CD27(-)) T cells and of activated CD11c(+)CD11b(high) cells to the lungs of aerosol-infected mice. For the first time, using in vivo analysis of transcriptome of inflammatory cytokines and chemokines of lung interstitial CD11c(+) cells, we show that in a low-dose aerosol infection model, BCG::RDI triggered an activation/inflammation program comparable to that induced by H37Rv while parental BCG, due to its overattenuation, did not initiate the activation program in lung interstitial CD11c(+) cells. Thus, products encoded by the ESAT-6 secretion system 1 of M. tuberculosis profoundly modify the interaction between mycobacteria and the host innate and adaptive immune system. These modifications can explain the previously described improved protective capacity of BCG::RDI vaccine candidate against M. tuberculosis challenge. The Journal of Immunology, 2005, 174: 3570-3579.

L8 ANSWER 7 OF 18 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 6
AN 2005:437506 BIOSIS
DN PREV200510226681
TI Immunogenic membrane-associated proteins of Mycobacterium tuberculosis revealed by proteomics.
AU Sinha, Sudhir [Reprint Author]; Kosalai, K.; Arora, Shalini; Namane, Abdelkader; Sharma, Pawan; Gaikwad, Anil N.; Brodin, Priscille; Cole, Stewart T.
CS Cent Drug Res Inst, Div Drug Target Discovery and Dev, Biochem Block,POB 173, Lucknow 226001, Uttar Pradesh, India
sinhas@lycos.com
SO Microbiology (Reading), (JUL 2005) Vol. 151, No. Part 7, pp. 2411-2419. ISSN: 1350-0872.
DT Article
LA English
ED Entered STN: 26 Oct 2005
Last Updated on STN: 26 Oct 2005
AB Membrane-associated proteins of Mycobacterium tuberculosis offer a challenge, as well as an opportunity, in the quest for better therapeutic

and prophylactic interventions against tuberculosis. The authors have previously reported that extraction with the detergent Triton X-114 (TX-114) is a useful step in proteomic analysis of mycobacterial cell membranes, and detergent-soluble membrane proteins of mycobacteria are potent stimulators of human T cells. In this study 1-D and 2-D gel electrophoresis-based protocols were used for the analysis of proteins in the TX-114 extract of M. tuberculosis membranes. Peptide mass mapping (using MALDI-TOF-MS, matrix assisted laser desorption/ionization time of flight mass spectrometry) of 116 samples led to the identification of 105 proteins, 9 of which were new to the M. tuberculosis proteome. Functional orthologues of 73 of these proteins were also present in Mycobacterium leprae, suggesting their relative importance. Bioinformatics predicted that as many as 73% of the proteins had a hydrophobic disposition. 1-D gel electrophoresis revealed more hydrophobic/transmembrane, and basic proteins than 2-D gel electrophoresis. Identified proteins fell into the following major categories: protein synthesis, cell wall biogenesis/architecture and conserved hypotheticals/unknowns. To identify immunodominant proteins of the detergent phase (DP), 14 low-molecular-mass fractions prepared by continuous-elution gel electrophoresis were subjected to T cell activation assays using blood samples from BCG -vaccinated healthy donors from a tuberculosis endemic area. Analysis of the responses (cell proliferation and IFN-gamma production) showed that the immunodominance of certain IDP fractions was most probably due to ribosomal proteins, which is consistent with both their specificity for mycobacteria and their abundance. Other membrane-associated proteins, including transmembrane proteins/lipoproteins and ESAT-6, did not appear to contribute significantly to the observed T cell responses.

L8 ANSWER 8 OF 18 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 7
AN 2005:1076707 CAPLUS
DN 145:44411
TI Tuberculosis: From genome to vaccine
AU de Jonge, Marien I.; Brosch, Roland; Brodin, Priscille; Demangel, Caroline; Cole, Stewart T.
CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, Paris, 75724, Fr.
SO Expert Review of Vaccines (2005), 4(4), 541-551
CODEN: ERVXAX; ISSN: 1476-0584
PB Future Drugs Ltd.
DT Journal; General Review
LA English
AB A review. The availability of mycobacterial genome sequences has paved the way to identifying potential tuberculosis vaccine candidates in order to replace the currently used bacillus Calmette-Guerin (BCG) vaccines that show variable protective efficacy in adults. Genomics provides the basis for bioinformatic, transcriptomic and proteomic anal., increases screening efficiency and enables valuable information concerning the biol. and virulence of the mycobacterial species to be extracted by comparative genomics. Although in silico results must be confirmed in vitro and in vivo, bioinformatic anal. of the genomes is highlighting candidates for testing. For designing subunit vaccines, attenuated or improved recombinant whole-cell live vaccines, information from the genomes of the human host and pathogenic mycobacterial species is of great help.
RE.CNT 111 THERE ARE 111 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 9 OF 18 CAPLUS COPYRIGHT 2007 ACS on STN
AN 2004:1050152 CAPLUS
DN 142:107880
TI Introduction to functional genomics of the Mycobacterium tuberculosis complex
AU Brodin, Priscille; Demangel, Caroline; Cole, Stewart T.
CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, Paris,

75724, Fr.
SO Tuberculosis and the Tubercle Bacillus (2005), 143-153. Editor(s): Cole, Stewart T. Publisher: American Society for Microbiology, Washington, D. C. CODEN: 69GFRV; ISBN: 1-55581-295-3
DT Conference; General Review
LA English
AB A review describes the three complete genome sequences of Mycobacterium tuberculosis H37Rv, M. tuberculosis CDC 1551, and M. bovis AF2122/97 and highlights the genomic differences between members of the M. tuberculosis complex. Emphasis is given to the comparison between the human pathogenic strains and the two vaccine strains, M. bovis bacille Calmette Guerin and M. microti. The application of functional genomic strategies, such as transcriptomics and transposon mutagenesis, to discover essential genes and to identify the function of the unknown open reading frames is also discussed. Finally, proteomics and structural genomics approaches, which have been made possible as a result of genomics, are discussed briefly.
RE.CNT 62 THERE ARE 62 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 10 OF 18 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 8
AN 2005:205775 BIOSIS
DN PREV200500205883
TI Evaluation of vaccines in the EU TB vaccine cluster using a guinea pig aerosol infection model of tuberculosis.
AU Williams, Ann [Reprint Author]; Hatch, Graham J.; Clark, Simon O.; Gooch, Karen E.; Hatch, Kim A.; Hall, Graham A.; Huygen, Kris; Ottenhoff, Tom H. M.; Franken, Kees L. M. C.; Andersen, Peter; Doherty, T. Mark; Kaufmann, Stefan H. E.; Grode, Leander; Seiler, Peter; Martin, Carlos; Gicquel, Brigitte; Cole, Stewart T.; Brodin, Priscille; Pym, Alexander S.; Dalemans, Wilfried; Cohen, Joe; Lobet, Yves; Goonetilleke, Nilu; McShane, Helen; Hill, Adrian; Parish, Tanya; Smith, Debbie; Stoker, Neil G.; Lowrie, Douglas B.; Kallenius, Gunilla; Svenson, Stefan; Pawowski, Andrzej; Blake, Karen; Marsh, Philip D.
CS Hlth Protect Agcy, Porton Down, Salisbury, Wilts, SP4 0JG, UK
ann.williams@camr.org.uk
SO Tuberculosis (Amsterdam), (January 2005) Vol. 85, No. 1-2, pp. 29-38. print.
ISSN: 1472-9792 (ISSN print).
DT Article
LA English
ED Entered STN: 1 Jun 2005
Last Updated on STN: 1 Jun 2005
AB The TB Vaccine Cluster project funded by the EU Fifth Framework programme aims to provide novel vaccines against tuberculosis that are suitable for evaluation in humans. This paper describes the studies of the protective efficacy of vaccines in a guinea pig aerosol-infection model of primary tuberculosis. The objective was to conduct comparative evaluations of vaccines that had previously demonstrated efficacy in other animal models. Groups of 6 guinea pigs were immunized with vaccines provided by the relevant EU Vaccine Cluster partners. Survival over 17 or 26 weeks was used as the principal measure of vaccine efficacy following aerosol challenge with H37Rv. Counts of mycobacteria in lungs and spleens, and histopathological changes in the lungs, were also used to provide evidence of protection. A total of 24 vaccines were evaluated in 4 experiments each of a different design. A heterologous prime-boost strategy of DNA and MVA, each expressing Ag85A and a fusion protein of ESAT-6 and Ag85B in adjuvant, protected the guinea pigs to the same extent as BCG. Genetically modified BCG vaccines and boosted BCG strategies also protected guinea pigs to the same extent as BCG but not statistically significantly better. A relatively high aerosol-challenge dose and evaluation over a protracted time post-challenge allowed superior protection over BCG to be

demonstrated by BCG boosted with MVA and fowl pox vectors expressing Ag85A. Copyright 2004 Elsevier Ltd. All rights reserved.

L8 ANSWER 11 OF 18 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 9

AN 2004:438631 BIOSIS

DN PREV200400437455

TI Cell envelope protein PPE68 contributes to Mycobacterium tuberculosis RDI immunogenicity independently of a 10-kilodalton culture filtrate protein and ESAT-6.

AU Demangel, Caroline [Reprint Author]; Brodin, Priscille; Cockle, Paul J.; Brosch, Roland; Majlessi, Laleh; Leclerc, Claude; Cole, Stewart T.

CS Unite Genet Mol Bacterienne, Inst Pasteur, 28 Rue Dr Roux, F-75724, Paris, 15, France
demangel@pasteur.fr

SO Infection and Immunity, (April 2004) Vol. 72, No. 4, pp. 2170-2176. print.
ISSN: 0019-9567 (ISSN print).

DT Article

LA English

ED Entered STN: 17 Nov 2004
Last Updated on STN: 17 Nov 2004

AB The protective efficacy of Mycobacterium bovis BCG can be markedly augmented by stable integration of Mycobacterium tuberculosis genomic region RD1. BCG complemented with RD1 (BCG ::RDI) encodes nine additional proteins. Among them, 10-kDa culture filtrate protein (CFP-10) and ESAT-6 (6-kDa early secreted antigenic target) are low-molecular-weight proteins that induce potent Th1 responses. Using pools of synthetic peptides, we have examined the potential immunogenicity of four other RD1 products (PE35, PPE68, Rv3878, and Rv3879c). PPE68, the protein encoded by rv3873, was the only one to elicit gamma interferon (IFN-gamma)-producing cells in C57BL/6 mice infected with M. tuberculosis. Anti-PPE68 T cells were predominantly raised against an epitope mapped in the N-terminal end of the protein. Importantly, inactivation of rv3873 in BCG::RD1 did not modify CFP-10 and ESAT-6 secretion. Moreover, the generation of IFN-gamma responses to these antigens following immunization with BCG::RD1 was independent of PPE68 expression. Taken together, these results show that PPE68 is an immunogenic product of the RD1 region, which does not interfere with the secretion and immunogenicity of CFP-10 and ESAT-6.

L8 ANSWER 12 OF 18 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 10

AN 2004:164676 BIOSIS

DN PREV200400168448

TI Macro-array and bioinformatic analyses reveal mycobacterial 'core' genes, variation in the ESAT-6 gene family and new phylogenetic markers for the Mycobacterium tuberculosis complex.

AU Marmiesse, Magali; Brodin, Priscille; Buchrieser, Carmen; Gutierrez, Christina; Simoes, Nathalie; Vincent, Veronique; Glaser, Philippe; Cole, Stewart T.; Brosch, Roland [Reprint Author]

CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, 25-28 Rue du Docteur Roux, 75724, Paris Cedex 15, France
rbrosch@pasteur.fr

SO Microbiology (Reading), (February 2004) Vol. 150, No. 2, pp. 483-496. print.
ISSN: 1350-0872 (ISSN print).

DT Article

LA English

ED Entered STN: 24 Mar 2004
Last Updated on STN: 24 Mar 2004

AB To better understand the biology and the virulence determinants of the two major mycobacterial human pathogens Mycobacterium tuberculosis and Mycobacterium leprae, their genome sequences have been determined

recently. In silico comparisons revealed that among the 1439 genes common to both M. tuberculosis and M. leprae, 219 genes code for proteins that show no similarity with proteins from other organisms. Therefore, the latter 'core' genes could be specific for mycobacteria or even for the intracellular mycobacterial pathogens. To obtain more information as to whether these genes really were mycobacteria-specific, they were included in a focused macro-array, which also contained genes from previously defined regions of difference (RD) known to be absent from Mycobacterium bovis BCG relative to M. tuberculosis. Hybridization of DNA from 40 strains of the M. tuberculosis complex and in silico comparison of these genes with the near-complete genome sequences from Mycobacterium avium, Mycobacterium marinum and Mycobacterium smegmatis were undertaken to answer this question. The results showed that among the 219 conserved genes, very few were not present in all the strains tested. Some of these missing genes code for proteins of the ESAT-6 family, a group of highly immunogenic small proteins whose presence and number is variable among the genomically highly conserved members of the M. tuberculosis complex. Indeed, the results suggest that, with few exceptions, the 'core' genes conserved among M. tuberculosis H37Rv and M. leprae are also highly conserved among other mycobacterial strains, which makes them interesting potential targets for developing new specific anti-mycobacterial drugs. In contrast, the genes from RD regions showed great variability among certain members of the M. tuberculosis complex, and some new specific deletions in Mycobacterium canettii, Mycobacterium microti and seal isolates were identified and further characterized during this study. Together with the distribution of a particular 6 or 7 bp micro-deletion in the gene encoding the polyketide synthase pks15/1, these results confirm and further extend the revised phylogenetic model for the M. tuberculosis complex recently presented.

L8 ANSWER 13 OF 18 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 11

AN 2004:326990 BIOSIS

DN PREV200400328635

TI Enhanced protection against tuberculosis by vaccination with recombinant Mycobacterium microti vaccine that induces T cell immunity against region of difference 1 antigens.

AU Brodin, Priscille; Majlessi, Laleh; Brosch, Roland; Smith, Debbie; Bancroft, Gregory; Clark, Simon; Williams, Ann; Leclerc, Claude; Cole, Stewart T. [Reprint Author]

CS Unite Genet Mol Bacterienne, Inst Pasteur, 28 Rue Docteur Roux, F-75724, Paris, 15, France
stcole@pasteur.fr

SO Journal of Infectious Diseases, (July 1 2004) Vol. 190, No. 1, pp. 115-122. print.
CODEN: JIDIAQ. ISSN: 0022-1899.

DT Article

LA English

ED Entered STN: 29 Jul 2004
Last Updated on STN: 29 Jul 2004

AB Mycobacterium microti, the vole bacillus, which was used as a live vaccine against tuberculosis until the 1970s, confers the same protection in humans as does Mycobacterium bovis bacille Calmette-Guerin (BCG). However, because the efficacy of the BCG vaccine varies considerably, we have tried to develop a better vaccine by reintroducing into M. microti the complete region of difference 1 (RD1), which is required for secretion of the potent T cell antigens early secreted antigen target (ESAT)-6 and culture filtrate protein (CFP)-10. The resultant recombinant strain, M. microti OV254::RD1-2F9, induced specific ESAT-6 and CFP-10 immune responses in mice with CD8+ T lymphocytes that had strong expression of the CD44hi activation marker. This vaccine also displayed better efficacy against disseminated disease in the mouse and the guinea pig models of tuberculosis than was seen in animals vaccinated with M. microti

alone or with BCG. The M. microti OV254::RD1-2F9 vaccine was less virulent and persistent in mice and than was BCG ::RD1-2F9 may represent a safer alternative to BCG::RD1-2F9.

L8 ANSWER 14 OF 18 CAPLUS COPYRIGHT 2007 ACS on STN
AN 2003:791413 CAPLUS
DN 139:304478
TI Identification of virulence associated regions RD1 and RD5 enabling the development of improved vaccines of M. bovis BCG and M. microti
IN Cole, Stewart; Pym, Alexander S.; Brosch, Roland; Brodin, Priscille; Majlessi, Laleh; Leclerc, Claude
PA Institut Pasteur, Fr.
SO Eur. Pat. Appl., 58 pp.
CODEN: EPXXDW
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 1350839	A1	20031008	EP 2002-290864	20020405
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
	CA 2481318	A1	20031016	CA 2003-2481318	20030401
	WO 2003085098	A2	20031016	WO 2003-IB1789	20030401
	WO 2003085098	A3	20040129		
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
	AU 2003223039	A1	20031020	AU 2003-223039	20030401
	EP 1492867	A2	20050105	EP 2003-719008	20030401
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
	US 2005220811	A1	20051006	US 2004-510021	20041001
PRAI	EP 2002-290864	A	20020405		
	WO 2003-IB1789	W	20030401		

AB The present invention relates to a strain of Mycobacterium bovis BCG or Mycobacterium microti, wherein said strain has integrated part or all of the RD1 region responsible for enhanced immunogenicity of the tubercle bacilli, especially the ESAT-6 and CFP-10 genes. These strains will be referred as the M. bovis BCG::RD1 or M. microti::RD1 strains and are useful as a new improved vaccine for preventing tuberculosis and as a therapeutical product enhancing the stimulation of the immune system for the treatment of bladder cancer.
RE.CNT 11 THERE ARE 11 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 15 OF 18 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 12
AN 2004:64037 BIOSIS
DN PREV200400065524
TI CD8+-T-cell responses of mycobacterium-infected mice to a newly identified major histocompatibility complex class I-restricted epitope shared by proteins of the ESAT-6 family.
AU Majlessi, Laleh [Reprint Author]; Rojas, Marie-Jesus; Brodin, Priscille; Leclerc, Claude
CS Unite de Biologie des Regulations Immunitaires, Institut Pasteur, 25, Rue

du Docteur Roux, 75724, Paris Cedex 15, France
lmajless@pasteur.fr
SO Infection and Immunity, (December 2003) Vol. 71, No. 12, pp. 7173-7177. print.
ISSN: 0019-9567 (ISSN print).
DT Article
LA English
ED Entered STN: 28 Jan 2004
Last Updated on STN: 28 Jan 2004
AB Here we describe the identification of a new CD8+-T-cell epitope, the GYAGTLQSL nonamer, shared by the TB10.3 and TB10.4 proteins of the Mycobacterium tuberculosis ESAT-6 family. Cytotoxic T cells from mycobacterium-infected mice efficiently recognized this epitope. GYAGTLQSL-specific T-cell hybridomas, which were able to recognize Mycobacterium bovis BCG-infected macrophages, were generated and now allow investigation of mycobacterial-antigen processing through the major histocompatibility complex class I pathway.

L8 ANSWER 16 OF 18 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 13
AN 2003:249243 BIOSIS
DN PREV200300249243
TI Recombinant BCG exporting ESAT-6 confers enhanced protection against tuberculosis.
AU Pym, Alexander S.; Brodin, Priscille; Majlessi, Laleh; Brosch, Roland; Demangel, Caroline; Williams, Ann; Griffiths, Karen E.; Marchal, Gilles; Leclerc, Claude; Cole, Stewart T. [Reprint Author]
CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, Paris, France
stcole@pasteur.fr
SO Nature Medicine, (May 2003) Vol. 9, No. 5, pp. 533-539. print.
ISSN: 1078-8956 (ISSN print).
DT Article
LA English
ED Entered STN: 28 May 2003
Last Updated on STN: 28 May 2003
AB The live tuberculosis vaccines Mycobacterium bovis BCG (bacille Calmette-Guerin) and Mycobacterium microti both lack the potent, secreted T-cell antigens ESAT-6 (6-kDa early secretory antigenic target) and CFP-10 (10-kDa culture filtrate protein). This is a result of independent deletions in the region of deletion-1 (RD1) locus, which is intact in virulent members of the Mycobacterium tuberculosis complex. To increase their immunogenicity and protective capacity, we complemented both vaccines with different constructs containing the esxA and esxB genes, which encode ESAT-6 and CFP-10 respectively, as well as a variable number of flanking genes. Only reintroduction of the complete locus, comprising at least 11 genes, led to full secretion of the antigens and resulted in specific ESAT-6-dependent immune responses; this suggests that the flanking genes encode a secretory apparatus. Mice and guinea pigs vaccinated with the recombinant strain BCG::RD1-2F9 were better protected against challenge with M. tuberculosis, showing less severe pathology and reduced dissemination of the pathogen, as compared with control animals immunized with BCG alone.

L8 ANSWER 17 OF 18 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 14
AN 2002:559623 BIOSIS
DN PREV200200559623
TI Bacterial artificial chromosome-based comparative genomic analysis identifies Mycobacterium microti as a natural ESAT-6 deletion mutant.
AU Brodin, Priscille; Eiglmeier, Karin; Marmiesse, Magali; Billault, Alain; Garnier, Thierry; Niemann, Stefan; Cole, Stewart T.; Brosch, Roland [Reprint author]

CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, 28 Rue du Docteur Roux, 75724, Paris Cedex 15, France
rbrosch@pasteur.fr
SO Infection and Immunity, (October, 2002) Vol. 70, No. 10, pp. 5568-5578. print.
CODEN: INFIBR. ISSN: 0019-9567.
DT Article
LA English
ED Entered STN: 30 Oct 2002
Last Updated on STN: 30 Oct 2002
AB Mycobacterium microti is a member of the Mycobacterium tuberculosis complex that causes tuberculosis in voles. Most strains of M. microti are harmless for humans, and some have been successfully used as live tuberculosis vaccines. In an attempt to identify putative virulence factors of the tubercle bacilli, genes that are absent from the avirulent M. microti but present in human pathogen M. tuberculosis or Mycobacterium bovis were searched for. A minimal set of 50 bacterial artificial chromosome (BAC) clones that covers almost all of the genome of M. microti OV254 was constructed, and individual BACs were compared to the corresponding BACs from M. bovis AF2122/97 and M. tuberculosis H37Rv. Comparison of pulsed-field gel-separated DNA digests of BAC clones led to the identification of 10 regions of difference (RD) between M. microti OV254 and M. tuberculosis. A 14-kb chromosomal region (RD1mic) that partly overlaps the RD1 deletion in the BCG vaccine strain was missing from the genomes of all nine tested M. microti strains. This region covers 13 genes, Rv3864 to Rv3876, in M. tuberculosis, including those encoding the potent ESAT-6 and CFP-10 antigens. In contrast, RD5mic, a region that contains three phospholipase C genes (plcA to -C), was missing from only the vole isolates and was present in M. microti strains isolated from humans . Apart from RD1mic and RD5mic other M. microti-specific deleted regions have been identified (MiD1 to MiD3). Deletion of MiD1 has removed parts of the direct repeat region in M. microti and thus contributes to the characteristic spoligotype of M. microti strains.

L8 ANSWER 18 OF 18 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 15
AN 2002:614464 BIOSIS
DN PREV200200614464
TI Loss of RD1 contributed to the attenuation of the live tuberculosis vaccines Mycobacterium bovis BCG and Mycobacterium microti.
AU Pym, Alexander S.; Brodin, Priscille; Brosch, Roland; Huerre, Michel; Cole, Stewart T. [Reprint author]
CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, 28 Rue du Docteur Roux, 75724, Paris Cedex 15, France
stcole@pasteur.fr
SO Molecular Microbiology, (November, 2002) Vol. 46, No. 3, pp. 709-717. print.
CODEN: MOMIEE. ISSN: 0950-382X.
DT Article
LA English
ED Entered STN: 4 Dec 2002
Last Updated on STN: 4 Dec 2002
AB Although large human populations have been safely immunized against tuberculosis with two live vaccines, Mycobacterium bovis BCG or Mycobacterium microti, the vole bacillus, the molecular basis for the avirulence of these vaccine strains remains unknown. Comparative genomics has identified a series of chromosomal deletions common to both virulent and avirulent species but only a single locus, RD1, that has been deleted from M. bovis BCG and M. microti. Restoration of RD1, by gene knock-in, resulted in a marked change in colonial morphology towards that of virulent tubercle bacilli. Three RD1-encoded

proteins were localized in the cell wall, and two of them, the immunodominant T-cell antigens ESAT-6 and CFP-10, were also found in culture supernatants. The BCG::RD1 and M. microti ::RD1 knock-ins grew more vigorously than controls in immunodeficient mice, inducing extensive splenomegaly and granuloma formation. Increased persistence and partial reversal of attenuation were observed when immunocompetent mice were infected with the BCG::RD1 knock-in, whereas BCG controls were cleared. Knocking-in five other RD loci did not affect the virulence of BCG. This study describes a genetic lesion that contributes to safety and opens new avenues for vaccine development.

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=> e majlessi laleh/au
E1            1     MAJLESSI KAMRAN REZA/AU
E2           32     MAJLESSI L/AU
E3           64 --> MAJLESSI LALEH/AU
E4            7     MAJLESSI M/AU
E5            3     MAJLESSI M R/AU
E6           14     MAJLESSI MEHRDAD/AU
E7            4     MAJLESSI MEHRDAD R/AU
E8           12     MAJLESSI N/AU
E9           12     MAJLESSI NAHID/AU
E10           2     MAJLESSI S A/AU
E11           5     MAJLESSIPOUR F/AU
E12           1     MAJLESSIPOUR FAE/AU

=> s e2-e3 and (bcg or microti)
L9           39 ("MAJLESSI L"/AU OR "MAJLESSI LALEH"/AU) AND (BCG OR MICROTI)

=> dup rem l9
PROCESSING COMPLETED FOR L9
L10          12 DUP REM L9 (27 DUPLICATES REMOVED)

=> d bib ab 1-
YOU HAVE REQUESTED DATA FROM 12 ANSWERS - CONTINUE? Y/(N):y

L10  ANSWER 1 OF 12  CAPLUS  COPYRIGHT 2007 ACS on STN
AN   2007:41383  CAPLUS
DN   146:140994
TI   Modified ESAT-6 derived from Mycobacterium tuberculosis and Mycobacterium
     leprae as vaccines for inducing interferon γ response to ESAT-6
     and/or CFP-10 against infection
IN   Brosch, Roland; Brodin, Priscille; Cole, Stewart; Majlessi, Laleh
     ; Leclerc, Claude
PA   Fr.
SO   U.S. Pat. Appl. Publ., 64pp.
     CODEN: USXXCO
DT   Patent
LA   English
FAN.CNT 1
     PATENT NO.          KIND   DATE        APPLICATION NO.         DATE
     --------------      ----   --------    --------------------    --------
PI   US 2007009547        A1    20070111    US 2006-455929          20060620
     WO 2007010413        A2    20070125    WO 2006-IB2884          20060622
     WO 2007010413        A3    20070830
         W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH,
             CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD,
             GE, GH, GM, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP,
             KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN,
             MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU,
             SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG,
             US, UZ, VC, VN, ZA, ZM, ZW
         RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE,
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IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AP, EA, EP, OA

PRAI US 2005-692561P P 20050622

AB A genetically modified strain of M. tuberculosis or Mycobacterium bovis BCG is provided, wherein the genetically modified strain comprises at least one modified sequence comprising SEQ ID NO: 1, SEQ ID NO: 2, or both, having at least one mutation at T2, Q4, F8, A14, L28, L29, W43, G45, Q55, Q56, N66, M83, V90, M93, or F94. In a preferred embodiment, the mutation is at least one of T2H, Q4L, F8I, A14R, L28A, L29S, W43R, G45T, Y51, Q55I, Q56A, N66I, N66A, M83I, V90R, M93T, or F94Q. Similarly, the genetically modified strain may also secrete ESAT-6 with a a histidine tag, tetra-cysteine tag or FLAG-tag, a GFP-fusion, or a short truncation at the C-terminal end of less than 20 amino acids.

L10 ANSWER 2 OF 12 CAPLUS COPYRIGHT 2007 ACS on STN

AN 2007:788499 CAPLUS

DN 147:164307

TI Inhibition of phagosome maturation by mycobacteria does not interfere with presentation of mycobacterial antigens by MHC molecules

AU Majlessi, Laleh; Combaluzier, Benoit; Albrecht, Imke; Garcia, Jessica E.; Nouze, Clemence; Pieters, Jean; Leclerc, Claude

CS Unite de Regulation Immunitaire et Vaccinologie, Institut Pasteur, Paris, Fr.

SO Journal of Immunology (2007), 179(3), 1825-1833
CODEN: JOIMA3; ISSN: 0022-1767

PB American Association of Immunologists

DT Journal

LA English

AB Pathogenic mycobacteria escape host innate immune responses by surviving within phagosomes of host macrophages and blocking their delivery to lysosomes. Avoiding lysosomal delivery may also be involved in the capacity of living mycobacteria to modulate MHC class I- or II-dependent T cell responses, which may contribute to their pathogenicity in vivo. In this study, the authors show that the presentation of mycobacterial Ags is independent of the site of intracellular residence inside professional APCs. Infection of mouse macrophages or dendritic cells in vitro with mycobacterial mutants that are unable to escape lysosomal transfer resulted in an identical efficiency of Ag presentation compared with wild-type mycobacteria. Moreover, in vivo, such mutants induced CD4+ Th1 or CD8+ CTL responses in mice against various mycobacterial Ags that were comparable to those induced by their wild-type counterparts. These results suggest that the limiting factor for the generation of an adaptive immune response against mycobacteria is not the degree of lysosomal delivery. These findings are important in the rational design of improved vaccines to combat mycobacterial diseases.

RE.CNT 57 THERE ARE 57 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 3 OF 12 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 1

AN 2006:351223 BIOSIS

DN PREV200600347024

TI High frequency of CD4(+) T cells specific for the TB10.4 protein correlates with protection against Mycobacterium tuberculosis infection.

AU Hervas-Stubbs, Sandra; Majlessi, Laleh; Simsova, Marcela; Morova, Jana; Rojas, Marie-Jesus; Nouze, Clemence; Brodin, Priscille; Sebo, Peter; Leclerc, Claude [Reprint Author]

CS Inst Pasteur, INSERM, E352, 25 Rue Docteur Roux, F-75724 Paris 15, France
cleclerc@pasteur.fr

SO Infection and Immunity, (JUN 2006) Vol. 74, No. 6, pp. 3396-3407.
CODEN: INFIBR. ISSN: 0019-9567.

DT Article

LA English
ED Entered STN: 12 Jul 2006
Last Updated on STN: 12 Jul 2006
AB TB10.4 is a newly identified antigen of Mycobacterium tuberculosis recognized by human and murine T cells upon mycobacterial infection. Here, we show that immunization with Mycobacterium bovis BCG induces a strong, genetically controlled, Th1 immune response against TB10.4 in mice. BALB/c and C57BL/6 strains behave as high and low responders to TB10.4 protein, respectively. The TB10.4:74-88 peptide was identified as an immunodominant CD4(+) T-cell epitope for H-2(d) mice. Since recent results, as well as the present study, have raised interest in TB10.4 as a subunit vaccine, we analyzed immune responses induced by this antigen delivered by a new vector, the adenylate cyclase (CyaA) of Bordetella pertussis. CyaA is able to target dendritic cells and to deliver CD4(+) or CD8(+) T-cell epitopes to the major histocompatibility complex class II/I molecule presentation pathways, triggering specific Th1 or cytotoxic T-lymphocyte (CTL) responses. Several CyaA harboring either the entire TB10.4 protein or various subfragments containing the TB10.4:20-28 CTL epitope were shown to induce TB10.4-specific Th1 CD4(+) and CD8(+) T-cell responses. However, none of the recombinant CyaA, injected in the absence of adjuvant, was able to induce protection against M. tuberculosis infection. In contrast, TB10.4 protein administered with a cocktail of strong adjuvants that triggered a strong Th1 CD4(+) T-cell response induced significant protection against M. tuberculosis challenge. These results confirm the potential value of the TB10.4 protein as a candidate vaccine and show that the presence of high frequencies of CD4(+) T cells specific to this strong immunogen correlates with protection against M. tuberculosis infection.

L10 ANSWER 4 OF 12 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 2
AN 2006:370142 BIOSIS
DN PREV200600369174
TI An increase in antimycobacterial Th1-cell responses by prime-boost protocols of immunization does not enhance protection against tuberculosis.
AU Majlessi, Laleh [Reprint Author]; Simsova, Marcela; Jarvis, Zdenka; Brodin, Priscille; Rojas, Marie-Jesus; Bauche, Cecile; Nouze, Clemence; Ladant, Daniel; Cole, Stewart T.; Sebo, Peter; Leclerc, Claude
CS Inst Pasteur, Unite Biol Regulat Immunitaries, INSERM, 25 Rue Dr Roux,E 352, F-75724 Paris 15, France
lmajless@pasteur.fr
SO Infection and Immunity, (APR 2006) Vol. 74, No. 4, pp. 2128-2137.
CODEN: INFIBR. ISSN: 0019-9567.
DT Article
LA English
ED Entered STN: 26 Jul 2006
Last Updated on STN: 26 Jul 2006
AB Bordetella pertussis adenylate cyclase (CyaA) toxoid is a powerful nonreplicative immunization vector targeting dendritic cells, which has already been used successfully in prophylactic and therapeutic vaccination in various preclinical animal models. Here, we investigated the potential of CyaA, harboring strong mycobacterial immunogens, i.e., the immunodominant regions of antigen 85A or the complete sequence of the 6-kDa early secreted antigenic target (ESAT-6) protein, to induce anti mycobacterial immunity. By generating T-cell hybridomas or by using T cells from mice infected with mycobacteria, we first demonstrated that the in vitro delivery of 85A or ESAT-6 to antigen-presenting cells by CyaA leads to processing and presentation, by major histocompatibility complex class II molecules, of the same epitopes as those displayed upon mycobacterial infection. Importantly, compared to the recombinant protein alone, the presentation of ESAT-6 in vitro was 100 times more efficient upon its delivery to antigen-presenting cells in fusion to CyaA. Immunization with CyaA-85A or CyaA-ESAT-6 in the absence of any adjuvant

induced strong antigen-specific lymphoproliferative, interleukin-2 (IL-2) and gamma interferon (IFN-gamma) cytokine responses, in the absence of any IL-4 or IL-5 production. When used as boosters after priming with a BCG expressing ESAT-6, the CyaA-85A and CyaA-ESAT-6 proteins were able to strikingly increase the sensitivity and intensity of proliferative and Th1-polarized responses and notably the frequency of antigen-specific IFN-gamma-producing CD4(+) T cells. However, immunization with these CyaA constructs as subunit vaccines alone or as boosters did not allow induction or improvement of protection against Mycobacterium tuberculosis infection. These results question the broadly admitted correlation between the frequency of IFN-gamma-producing CD4(+) T cells and the level of protection against tuberculosis.

L10 ANSWER 5 OF 12 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 3
AN 2006:176683 BIOSIS
DN PREV200600166449
TI Dissection of ESAT-6 system 1 of Mycobacterium tuberculosis and impact on immunogenicity and virulence.
AU Brodin, Priscille; Majlessi, Laleh; Marsollier, Laurent; de Jonge, Marien I.; Bottai, Daria; Demangel, Caroline; Hinds, Jason; Neyrolles, Olivier; Butcher, Philip D.; Leclerc, Claude; Cole, Stewart T.; Brosch, Roland [Reprint Author]
CS Inst Pasteur, Unite Genet Mol Bacterienne, 25-28 Rue Docteur Roux, F-75724 Paris 15, France
rbrosch@pasteur.fr
SO Infection and Immunity, (JAN 2006) Vol. 74, No. 1, pp. 88-98.
CODEN: INFIBR. ISSN: 0019-9567.
DT Article
LA English
ED Entered STN: 9 Mar 2006
Last Updated on STN: 9 Mar 2006
AB The dedicated secretion system ESX-1 of Mycobacterium tuberculosis encoded by the extended RD1 region (extRD1) assures export of the ESAT-6 protein and its partner, the 10-kDa culture filtrate protein CFP-10, and is missing from the vaccine strains M. bovis BCG and M. microti. Here, we systematically investigated the involvement of each individual ESX-1 gene in the secretion of both antigens, specific immunogenicity, and virulence. ESX-1-complemented BCG and M. microti strains were more efficiently engulfed by bone-marrow-derived macrophages than controls, and this may account for the enhanced in vivo growth of ESX-1-carrying strains. Inactivation of gene pe35 (Rv3872) impaired expression of CFP-10 and ESAT-6, suggesting a role in regulation. Genes Rv3868, Rv3869, Rv3870, Rv3871, and Rv3877 encoding an ATP-dependent chaperone and translocon were essential for secretion of ESAT-6 and CFP-10 in contrast to ppe68 Rv3873 and Rv3876, whose inactivation did not impair secretion of ESAT-6. A strict correlation was found between ESAT-6 export and the generation of ESAT-6 specific T-cell responses in mice. Furthermore, ESAT-6 secretion and specific immunogenicity were almost always correlated with enhanced virulence in the SCID mouse model. Only loss of Rv3865 and part of Rv3866 did not affect ESAT-6 secretion or immunogenicity but led to attenuation. This suggests that Rv3865/66 represent a new virulence factor that is independent from ESAT-6 secretion. The present study has allowed us to identify new aspects of the extRD1 region of M. tuberculosis and to explore its role in the pathogenesis of tuberculosis.

L10 ANSWER 6 OF 12 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 4
AN 2006:7638 BIOSIS
DN PREV200600007409
TI Functional analysis of early secreted antigenic target-6, the dominant T-cell antigen of Mycobacterium tuberculosis, reveals key residues involved in secretion, complex formation, virulence, and immunogenicity.

AU Brodin, Priscille; de Jonge, Marien I.; Majlessi, Laleh; Leclerc, Claude; Nilges, Michael; Cole, Stewart T.; Brosch, Roland [Reprint Author]

CS Inst Pasteur, Unite Genet Mol Bacterienne, 28 Rue Dr Roux, F-75724 Paris, France
rbrosch@pasteur.fr

SO Journal of Biological Chemistry, (OCT 7 2005) Vol. 280, No. 40, pp. 33953-33959.
CODEN: JBCHA3. ISSN: 0021-9258.

DT Article

LA English

ED Entered STN: 14 Dec 2005
Last Updated on STN: 14 Dec 2005

AB Proteins of the 6-kDa early secreted antigenic target (ESAT-6) secretion system-1 of Mycobacterium tuberculosis are not only strongly involved in the anti-mycobacterial Th1-host immune response but are also key players for virulence. In this study, protein engineering together with bioinformatic, immunological, and virulence analyses allowed us to pinpoint regions of the ESAT-6 molecule that are critical for its biological activity in M. tuberculosis. Mutation of the Trp-Xaa-Gly motif, conserved in a wide variety of ESAT-6-like proteins, abolished complex formation with the partner protein CFP-10, induction of specific T-cell responses, and virulence. Replacement of conserved Leu residues interfered with secretion, coiled-coil formation, and virulence, whereas certain mutations at the extreme C terminus did not affect secretion but caused attenuation, possibly because of altered ESAT-6 targeting or trafficking. In contrast, the mutation of several residues on the outer surface of the four-helical bundle structure of the ESAT-6 center dot CFP-10 complex showed much less effect. Construction of recombinant BCG expressing ESAT-6 with a C-terminal hexahistidine tag allowed us to co-purify ESAT-6 and CFP-10, experimentally confirming their strong interaction both in and outside of the mycobacterial cell. The strain induced potent, antigen-specific T-cell responses and intermediate in vivo growth in mice, suggesting that it remained immunogenic and biologically active despite the tag. Together with previous NMR data, the results of this study have allowed a biologically relevant model of the ESAT-6 center dot CFP-10 complex to be constructed that is critical for understanding the structure-function relationship in tuberculosis pathogenesis.

L10 ANSWER 7 OF 12 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 5

AN 2005:215081 BIOSIS

DN PREV200510005668

TI Influence of ESAT-6 secretion system 1 (RD1) of Mycobacterium tuberculosis on the interaction between mycobacteria and the host immune system.

AU Majlessi, Laleh [Reprint Author]; Brodin, Priscille; Brosch, Roland; Rojas, Marie-Jesus; Khun, Huot; Huerre, Michel; Cole, Stewart T.; Leclerc, Claude

CS Inst Pasteur, Unite Biol Regulat Immun, INSERM, Equipe 352, 25,Rue Dr Roux, F-75724 Paris 15, France
lmajless@pasteur.fr

SO Journal of Immunology, (MAR 15 2005) Vol. 174, No. 6, pp. 3570-3579.
CODEN: JOIMA3. ISSN: 0022-1767.

DT Article

LA English

ED Entered STN: 10 Jun 2005
Last Updated on STN: 10 Jun 2005

AB The chromosomal locus encoding the early secreted antigenic target, 6 kDa (ESAT-6) secretion system I of Mycobacterium tuberculosis, also referred to as "region of difference I (RD1)," is absent from Mycobacterium bovis bacillus Calmette-Guerin (BCG). In this study, using low-dose aerosol infection in mice, we demonstrate that BCG complemented with RD1 (BCG::RD1) displays markedly increased virulence which albeit does not attain that of M. tuberculosis H37Rv. Nevertheless,

phenotypic and functional analyses of immune cells at the site of infection show that the capacity of BCG::RD1 to initiate recruitment/activation of immune cells is compaiable to that of fully virulent H37Rv. Indeed, in contrast to the parental BCG, BCG::RDI mimics H37Rv and induces substantial influx of activated (CD44(high)CD45RB(-)CD62L(-)) or effector (CD45RB(-)CD27(-)) T cells and of activated CD11c(+)CD11b(high) cells to the lungs of aerosol-infected mice. For the first time, using in vivo analysis of transcriptome of inflammatory cytokines and chemokines of lung interstitial CD11c(+) cells, we show that in a low-dose aerosol infection model, BCG::RDI triggered an activation/inflammation program comparable to that induced by H37Rv while parental BCG, due to its overattenuation, did not initiate the activation program in lung interstitial CD11c(+) cells. Thus, products encoded by the ESAT-6 secretion system 1 of M. tuberculosis profoundly modify the interaction between mycobacteria and the host innate and adaptive immune system. These modifications can explain the previously described improved protective capacity of BCG::RDI vaccine candidate against M. tuberculosis challenge. The Journal of Immunology, 2005, 174: 3570-3579.

L10 ANSWER 8 OF 12 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 6
AN 2004:438631 BIOSIS
DN PREV200400437455
TI Cell envelope protein PPE68 contributes to Mycobacterium tuberculosis RDI immunogenicity independently of a 10-kilodalton culture filtrate protein and ESAT-6.
AU Demangel, Caroline [Reprint Author]; Brodin, Priscille; Cockle, Paul J.; Brosch, Roland; Majlessi, Laleh; Leclerc, Claude; Cole, Stewart T.
CS Unite Genet Mol Bacterienne, Inst Pasteur, 28 Rue Dr Roux, F-75724, Paris, 15, France
demangel@pasteur.fr
SO Infection and Immunity, (April 2004) Vol. 72, No. 4, pp. 2170-2176. print. ISSN: 0019-9567 (ISSN print).
DT Article
LA English
ED Entered STN: 17 Nov 2004
Last Updated on STN: 17 Nov 2004
AB The protective efficacy of Mycobacterium bovis BCG can be markedly augmented by stable integration of Mycobacterium tuberculosis genomic region RD1. BCG complemented with RD1 (BCG ::RDI) encodes nine additional proteins. Among them, 10-kDa culture filtrate protein (CFP-10) and ESAT-6 (6-kDa early secreted antigenic target) are low-molecular-weight proteins that induce potent Th1 responses. Using pools of synthetic peptides, we have examined the potential immunogenicity of four other RD1 products (PE35, PPE68, Rv3878, and Rv3879c). PPE68, the protein encoded by rv3873, was the only one to elicit gamma interferon (IFN-gamma)-producing cells in C57BL/6 mice infected with M. tuberculosis. Anti-PPE68 T cells were predominantly raised against an epitope mapped in the N-terminal end of the protein. Importantly, inactivation of rv3873 in BCG::RD1 did not modify CFP-10 and ESAT-6 secretion. Moreover, the generation of IFN-gamma responses to these antigens following immunization with BCG::RD1 was independent of PPE68 expression. Taken together, these results show that PPE68 is an immunogenic product of the RD1 region, which does not interfere with the secretion and immunogenicity of CFP-10 and ESAT-6.

L10 ANSWER 9 OF 12 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 7
AN 2004:326990 BIOSIS
DN PREV200400328635
TI Enhanced protection against tuberculosis by vaccination with recombinant Mycobacterium microti vaccine that induces T cell immunity

against region of difference 1 antigens.
AU Brodin, Priscille; Majlessi, Laleh; Brosch, Roland; Smith, Debbie; Bancroft, Gregory; Clark, Simon; Williams, Ann; Leclerc, Claude; Cole, Stewart T. [Reprint Author]
CS Unite Genet Mol Bacterienne, Inst Pasteur, 28 Rue Docteur Roux, F-75724, Paris, 15, France
stcole@pasteur.fr
SO Journal of Infectious Diseases, (July 1 2004) Vol. 190, No. 1, pp. 115-122. print.
CODEN: JIDIAQ. ISSN: 0022-1899.
DT Article
LA English
ED Entered STN: 29 Jul 2004
Last Updated on STN: 29 Jul 2004
AB Mycobacterium microti, the vole bacillus, which was used as a live vaccine against tuberculosis until the 1970s, confers the same protection in humans as does Mycobacterium bovis bacille Calmette-Guerin (BCG). However, because the efficacy of the BCG vaccine varies considerably, we have tried to develop a better vaccine by reintroducing into M. microti the complete region of difference 1 (RD1), which is required for secretion of the potent T cell antigens early secreted antigen target (ESAT)-6 and culture filtrate protein (CFP)-10. The resultant recombinant strain, M. microti OV254::RD1-2F9, induced specific ESAT-6 and CFP-10 immune responses in mice with CD8+ T lymphocytes that had strong expression of the CD44hi activation marker. This vaccine also displayed better efficacy against disseminated disease in the mouse and the guinea pig models of tuberculosis than was seen in animals vaccinated with M. microti alone or with BCG. The M. microti OV254::RD1-2F9 vaccine was less virulent and persistent in mice and than was BCG ::RD1-2F9 may represent a safer alternative to BCG::RD1-2F9.

L10 ANSWER 10 OF 12 CAPLUS COPYRIGHT 2007 ACS on STN
AN 2003:791413 CAPLUS
DN 139:304478
TI Identification of virulence associated regions RD1 and RD5 enabling the development of improved vaccines of M. bovis BCG and M. microti
IN Cole, Stewart; Pym, Alexander S.; Brosch, Roland; Brodin, Priscille; Majlessi, Laleh; Leclerc, Claude
PA Institut Pasteur, Fr.
SO Eur. Pat. Appl., 58 pp.
CODEN: EPXXDW
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI EP 1350839	A1	20031008	EP 2002-290864	20020405
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
CA 2481318	A1	20031016	CA 2003-2481318	20030401
WO 2003085098	A2	20031016	WO 2003-IB1789	20030401
WO 2003085098	A3	20040129		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				

AU 2003223039 A1 20031020 AU 2003-223039 20030401
EP 1492867 A2 20050105 EP 2003-719008 20030401
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK
US 2005220811 A1 20051006 US 2004-510021 20041001
PRAI EP 2002-290864 A 20020405
WO 2003-IB1789 W 20030401
AB The present invention relates to a strain of Mycobacterium bovis BCG or Mycobacterium microti, wherein said strain has integrated part or all of the RD1 region responsible for enhanced immunogenicity of the tubercle bacilli, especially the ESAT-6 and CFP-10 genes. These strains will be referred as the M. bovis BCG::RD1 or M. microti::RD1 strains and are useful as a new improved vaccine for preventing tuberculosis and as a therapeutical product enhancing the stimulation of the immune system for the treatment of bladder cancer.
RE.CNT 11 THERE ARE 11 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 11 OF 12 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 8
AN 2004:64037 BIOSIS
DN PREV200400065524
TI CD8+-T-cell responses of mycobacterium-infected mice to a newly identified major histocompatibility complex class I-restricted epitope shared by proteins of the ESAT-6 family.
AU Majlessi, Laleh [Reprint Author]; Rojas, Marie-Jesus; Brodin, Priscille; Leclerc, Claude
CS Unite de Biologie des Regulations Immunitaires, Institut Pasteur, 25, Rue du Docteur Roux, 75724, Paris Cedex 15, France
lmajless@pasteur.fr
SO Infection and Immunity, (December 2003) Vol. 71, No. 12, pp. 7173-7177. print.
ISSN: 0019-9567 (ISSN print).
DT Article
LA English
ED Entered STN: 28 Jan 2004
Last Updated on STN: 28 Jan 2004
AB Here we describe the identification of a new CD8+-T-cell epitope, the GYAGTLQSL nonamer, shared by the TB10.3 and TB10.4 proteins of the Mycobacterium tuberculosis ESAT-6 family. Cytotoxic T cells from mycobacterium-infected mice efficiently recognized this epitope. GYAGTLQSL-specific T-cell hybridomas, which were able to recognize Mycobacterium bovis BCG-infected macrophages, were generated and now allow investigation of mycobacterial-antigen processing through the major histocompatibility complex class I pathway.

L10 ANSWER 12 OF 12 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 9
AN 2003:249243 BIOSIS
DN PREV200300249243
TI Recombinant BCG exporting ESAT-6 confers enhanced protection against tuberculosis.
AU Pym, Alexander S.; Brodin, Priscille; Majlessi, Laleh; Brosch, Roland; Demangel, Caroline; Williams, Ann; Griffiths, Karen E.; Marchal, Gilles; Leclerc, Claude; Cole, Stewart T. [Reprint Author]
CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, Paris, France
stcole@pasteur.fr
SO Nature Medicine, (May 2003) Vol. 9, No. 5, pp. 533-539. print.
ISSN: 1078-8956 (ISSN print).
DT Article
LA English
ED Entered STN: 28 May 2003
Last Updated on STN: 28 May 2003

AB The live tuberculosis vaccines Mycobacterium bovis BCG (bacille Calmette-Guerin) and Mycobacterium microti both lack the potent, secreted T-cell antigens ESAT-6 (6-kDa early secretory antigenic target) and CFP-10 (10-kDa culture filtrate protein). This is a result of independent deletions in the region of deletion-1 (RD1) locus, which is intact in virulent members of the Mycobacterium tuberculosis complex. To increase their immunogenicity and protective capacity, we complemented both vaccines with different constructs containing the esxA and esxB genes, which encode ESAT-6 and CFP-10 respectively, as well as a variable number of flanking genes. Only reintroduction of the complete locus, comprising at least 11 genes, led to full secretion of the antigens and resulted in specific ESAT-6-dependent immune responses; this suggests that the flanking genes encode a secretory apparatus. Mice and guinea pigs vaccinated with the recombinant strain BCG::RD1-2F9 were better protected against challenge with M. tuberculosis, showing less severe pathology and reduced dissemination of the pathogen, as compared with control animals immunized with BCG alone.

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=> e demangel caroline/au
E1            1     DEMANGEAT R/AU
E2           57     DEMANGEL C/AU
E3           78 --> DEMANGEL CAROLINE/AU
E4            2     DEMANGEL CHANTAL/AU
E5            1     DEMANGEL J P/AU
E6            4     DEMANGEL L/AU
E7            1     DEMANGEON A/AU
E8            6     DEMANGEON FRANCIS/AU
E9            1     DEMANGEON M/AU
E10           1     DEMANGEON M YVON/AU
E11           2     DEMANGEON P/AU
E12           2     DEMANGEON PAUL/AU

=> s e2-e3 and (bcg or microti)
L11          53 ("DEMANGEL C"/AU OR "DEMANGEL CAROLINE"/AU) AND (BCG OR MICROTI)


=> dup rem l11
PROCESSING COMPLETED FOR L11
L12           15 DUP REM L11 (38 DUPLICATES REMOVED)

=> d bib ab 1-
YOU HAVE REQUESTED DATA FROM 15 ANSWERS - CONTINUE? Y/(N):y

L12  ANSWER 1 OF 15  BIOSIS  COPYRIGHT (c) 2007 The Thomson Corporation  on STN
     DUPLICATE 1
AN   2006:634647  BIOSIS
DN   PREV200600639875
TI   Towards an immunodiagnostic test for leprosy.
AU   Araoz, Romulo; Honore, Nadine; Banu, Sayera; Demangel, Caroline;
     Cissoko, Yakouba; Arama, Charles; Uddin, Mohammad Khaja Mafij; Hadi, S. K.
     Abdul; Monot, Marc; Cho, Sang-Nae; Ji, Baohong; Brennan, Patrick J.; Sow,
     Samba; Cole, Stewart T. [Reprint Author]
CS   Inst Pasteur, Unite Genet Mol Bacterienne, 28 Rue Dr Roux, F-75724 Paris
     15, France
     stcole@pasteur.fr
SO   Microbes and Infection, (JUL 2006) Vol. 8, No. 8, pp. 2270-2276.
     ISSN: 1286-4579.
DT   Article
LA   English
ED   Entered STN: 22 Nov 2006
     Last Updated on STN: 22 Nov 2006
AB   In addition to multidrug therapy, elimination of leprosy requires improved
     diagnostic methods. Using a comparative genomics approach, 17 potential
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protein antigens (MLP) that are restricted to Mycobacterium leprae, or of limited distribution, were produced and tested for antigen-specific immune responses on leprosy patients, healthy contacts of leprosy patients, and tuberculosis patients in Mali and Bangladesh, as well as on non-endemic controls. T-cell antigenicity of MLP was confirmed by IFN-gamma production in whole-blood assays with the highest responses observed in paucibacillary leprosy patients and healthy contacts. Four MLP behaved well in both countries and induced significantly different responses between the study groups. Peptides carrying T cell epitopes from one of the antigens gave promising results in restimulation assays in mice and immune responses were not influenced by prior exposure to BCG or environmental mycobacteria. This study provides the immunological framework for the development of a specific, peptide-based immunodiagnostic test for leprosy. (c) 2006 Elsevier SAS. All rights reserved.

L12 ANSWER 2 OF 15 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 2
AN 2006:634624 BIOSIS
DN PREV200600639852
TI Immunogenicity of Mycobacterium ulcerans Hsp65 and protective efficacy of a Mycobacterium leprae Hsp65-based DNA vaccine against Buruli ulcer.
AU Coutanceau, Emmanuelle; Legras, Pierre; Marsollier, Laurent; Reysset, Gilles; Cole, Stewart T.; Demangel, Caroline [Reprint Author]
CS Inst Pasteur, Unite Genet Mol Bacterienne, 25 Rue Dr Roux, F-75724 Paris 15, France
demangel@pasteur.fr
SO Microbes and Infection, (JUL 2006) Vol. 8, No. 8, pp. 2075-2081.
ISSN: 1286-4579.
DT Article
LA English
ED Entered STN: 22 Nov 2006
Last Updated on STN: 22 Nov 2006
AB Buruli ulcer, a disease caused by Mycobacterium ulcerans, is emerging as an increasingly important cause of morbidity throughout the world, for which surgery is the only efficient treatment to date. The aim of this work was to identify potential vaccine candidates in an experimental model of mouse infection. In BALB/c mice infected with M. ulcerans subcutaneously, Hsp65 appeared to be an immunodominant antigen eliciting both Immoral and cellular responses. However, vaccination of mice with a DNA vector encoding Mycobacterium leprae Hsp65 only poorly limited the progression of M. ulcerans infection. In contrast, a substantial degree of protection was conferred by subcutaneous vaccination with BCG , suggesting that BCG antigens that are conserved in M. ulcerans, such as TB 10.4, the 19 kDa antigen, PstS3 and Hsp70, may be interesting to consider as subunit vaccines in future prospects. (c) 2006 Elsevier SAS. All rights reserved.

L12 ANSWER 3 OF 15 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 3
AN 2006:176683 BIOSIS
DN PREV200600166449
TI Dissection of ESAT-6 system 1 of Mycobacterium tuberculosis and impact on immunogenicity and virulence.
AU Brodin, Priscille; Majlessi, Laleh; Marsollier, Laurent; de Jonge, Marien I.; Bottai, Daria; Demangel, Caroline; Hinds, Jason; Neyrolles, Olivier; Butcher, Philip D.; Leclerc, Claude; Cole, Stewart T.; Brosch, Roland [Reprint Author]
CS Inst Pasteur, Unite Genet Mol Bacterienne, 25-28 Rue Docteur Roux, F-75724 Paris 15, France
rbrosch@pasteur.fr
SO Infection and Immunity, (JAN 2006) Vol. 74, No. 1, pp. 88-98.
CODEN: INFIBR. ISSN: 0019-9567.
DT Article

LA English
ED Entered STN: 9 Mar 2006
Last Updated on STN: 9 Mar 2006
AB The dedicated secretion system ESX-1 of Mycobacterium tuberculosis encoded by the extended RD1 region (extRD1) assures export of the ESAT-6 protein and its partner, the 10-kDa culture filtrate protein CFP-10, and is missing from the vaccine strains M. bovis BCG and M. microti. Here, we systematically investigated the involvement of each individual ESX-1 gene in the secretion of both antigens, specific immunogenicity, and virulence. ESX-1-complemented BCG and M. microti strains were more efficiently engulfed by bone-marrow-derived macrophages than controls, and this may account for the enhanced in vivo growth of ESX-1-carrying strains. Inactivation of gene pe35 (Rv3872) impaired expression of CFP-10 and ESAT-6, suggesting a role in regulation. Genes Rv3868, Rv3869, Rv3870, Rv3871, and Rv3877 encoding an ATP-dependent chaperone and translocon were essential for secretion of ESAT-6 and CFP-10 in contrast to ppe68 Rv3873 and Rv3876, whose inactivation did not impair secretion of ESAT-6. A strict correlation was found between ESAT-6 export and the generation of ESAT-6 specific T-cell responses in mice. Furthermore, ESAT-6 secretion and specific immunogenicity were almost always correlated with enhanced virulence in the SCID mouse model. Only loss of Rv3865 and part of Rv3866 did not affect ESAT-6 secretion or immunogenicity but led to attenuation. This suggests that Rv3865/66 represent a new virulence factor that is independent from ESAT-6 secretion. The present study has allowed us to identify new aspects of the extRD1 region of M. tuberculosis and to explore its role in the pathogenesis of tuberculosis.

L12 ANSWER 4 OF 15 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 4
AN 2005:240405 BIOSIS
DN PREV200510029170
TI Differential effects of prior exposure to environmental mycobacteria on vaccination with Mycobacterium bovis BCG or a recombinant BCG strain expressing RD1 antigens.
AU Demangel, Caroline [Reprint Author]; Garnier, Thierry; Rosenkrands, Ida; Cole, Stewart T.
CS Inst Pasteur, Unite Genet Mol Bacterienne, 28 Rue Dr Roux, F-75724 Paris, France
demangel@pasteur.fr
SO Infection and Immunity, (APR 2005) Vol. 73, No. 4, pp. 2190-2196.
CODEN: INFIBR. ISSN: 0019-9567.
DT Article
LA English
ED Entered STN: 29 Jun 2005
Last Updated on STN: 29 Jun 2005
AB In silico analysis reveals that most protective antigens expressed by the antituberculous vaccine Mycobacterium bovis BCG (BCG) are conserved in M. avium, supporting the hypothesis that exposure to environmental mycobacteria generates cross-reactive immune responses blocking BCG activity. We investigated the impact of sensitization with M. avium, M. scrofulaceum, or M. vaccae on the protective efficacy of a recombinant BCG strain expressing RD1 antigens (BCG::RD1), using a mouse model of experimental tuberculosis (TB). No evidence that the RD1-encoded antigens ESAT-6, CFP-10, and PPE68 were expressed by these environmental strains could be demonstrated by Western blot analysis. Mice sensitized with each of these strains did not prime cellular immune responses cross-reacting with the immunodominant ESAT-6. Importantly, clearance of BCG::RD1 from the lungs and spleens of mice exposed to each of the environmental strains before vaccination was minimal compared to that of BCG. In mice sensitized with M. avium, increased persistence of BCG::RD1 correlated with stronger antimycobacterial gamma interferon responses and enhanced protection against aerosol infection with M. tuberculosis,

compared to BCG. In contrast, animals exposed to M. scrofulaceum or M. vaccae prior to vaccination with BCG or BCG::RD1 were better protected against TB than were the unsensitized controls. Our results suggest that the inhibitory effect of environmental mycobacteria on the protective efficacy of BCG depends critically on the extent of cross-recognition of antigens shared with the vaccine. In hosts sensitized with M. avium, potent immunogenicity of ESAT-6 and increased persistence of BCG::RD1 may allow this recombinant vaccine to overcome preexisting antimycobacterial responses.

L12 ANSWER 5 OF 15 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 5
AN 2005:430578 BIOSIS
DN PREV200510218487
TI Modulation of the host immune response by a transient intracellular stage of Mycobacterium ulcerans: the contribution of endogenous mycolactone toxin.
AU Coutanceau, Emmanuelle; Marsollier, Laurent; Brosch, Roland; Perret, Emmanuelle; Goossens, Pierre; Tanguy, Myriam; Cole, Stewart T.; Small, Pamela L. C.; Demangel, Caroline [Reprint Author]
CS Inst Pasteur, Unite Genet Mol Bacterienne, Paris, France
demangel@pasteur.fr
SO Cellular Microbiology, (AUG 2005) Vol. 7, No. 8, pp. 1187-1196.
ISSN: 1462-5814.
DT Article
LA English
ED Entered STN: 26 Oct 2005
Last Updated on STN: 26 Oct 2005
AB Mycobacterium ulcerans (Mu), the aetiological agent of Buruli ulcer, is an extracellular pathogen producing the macrolide toxin mycolactone. Using a mouse model of intradermal infection, we found that Mu was initially captured by phagocytes and transported to draining lymph nodes (DLN) within host cells. Similar to Buruli ulcers in humans, the infection site eventually became ulcerated with tissue necrosis and extracellular bacteria, at later stages. In contrast to Mycobacterium bovis BCG (BCG), Mu did not disseminate to the spleen. However, mice infected with Mu or BCG developed comparable primary cellular responses to mycobacterial antigens in DLN and spleen. The role of mycolactone in this sequence of events was examined with a mycolactone-deficient (mup045) mutant of Mu. Mup045 bacilli were better internalized than wild-type (wt) bacteria by mouse phagocytes in vitro. Moreover, infection with wt but not mup045 Mu led to inhibition of TNF-alpha expression, upregulation of MIP-2 chemokine, and host cell death within 1 day. Our results suggest that mycolactone expression during the intracellular life of Mu may contribute to immune evasion by inhibiting phagocytosis, provoking apoptosis of antigen presenting cells and altering the establishment of an appropriate inflammatory reaction.

L12 ANSWER 6 OF 15 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 6
AN 2005:1076707 CAPLUS
DN 145:44411
TI Tuberculosis: From genome to vaccine
AU de Jonge, Marien I.; Brosch, Roland; Brodin, Priscille; Demangel, Caroline; Cole, Stewart T.
CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, Paris, 75724, Fr.
SO Expert Review of Vaccines (2005), 4(4), 541-551
CODEN: ERVXAX; ISSN: 1476-0584
PB Future Drugs Ltd.
DT Journal; General Review
LA English
AB A review. The availability of mycobacterial genome sequences has paved the way to identifying potential tuberculosis vaccine candidates in order

to replace the currently used bacillus Calmette-Guerin (BCG) vaccines that show variable protective efficacy in adults. Genomics provides the basis for bioinformatic, transcriptomic and proteomic anal., increases screening efficiency and enables valuable information concerning the biol. and virulence of the mycobacterial species to be extracted by comparative genomics. Although in silico results must be confirmed in vitro and in vivo, bioinformatic anal. of the genomes is highlighting candidates for testing. For designing subunit vaccines, attenuated or improved recombinant whole-cell live vaccines, information from the genomes of the human host and pathogenic mycobacterial species is of great help.

RE.CNT 111 THERE ARE 111 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 7 OF 15 CAPLUS COPYRIGHT 2007 ACS on STN
AN 2004:1050152 CAPLUS
DN 142:107880
TI Introduction to functional genomics of the Mycobacterium tuberculosis complex
AU Brodin, Priscille; Demangel, Caroline; Cole, Stewart T.
CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, Paris, 75724, Fr.
SO Tuberculosis and the Tubercle Bacillus (2005), 143-153. Editor(s): Colè, Stewart T. Publisher: American Society for Microbiology, Washington, D. C. CODEN: 69GFRV; ISBN: 1-55581-295-3
DT Conference; General Review
LA English
AB A review describes the three complete genome sequences of Mycobacterium tuberculosis H37Rv, M. tuberculosis CDC 1551, and M. bovis AF2122/97 and highlights the genomic differences between members of the M. tuberculosis complex. Emphasis is given to the comparison between the human pathogenic strains and the two vaccine strains, M. bovis bacille Calmette Guerin and M. microti. The application of functional genomic strategies, such as transcriptomics and transposon mutagenesis, to discover essential genes and to identify the function of the unknown open reading frames is also discussed. Finally, proteomics and structural genomics approaches, which have been made possible as a result of genomics, are discussed briefly.

RE.CNT 62 THERE ARE 62 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 8 OF 15 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 7
AN 2004:438631 BIOSIS
DN PREV200400437455
TI Cell envelope protein PPE68 contributes to Mycobacterium tuberculosis RD1 immunogenicity independently of a 10-kilodalton culture filtrate protein and ESAT-6.
AU Demangel, Caroline [Reprint Author]; Brodin, Priscille; Cockle, Paul J.; Brosch, Roland; Majlessi, Laleh; Leclerc, Claude; Cole, Stewart T.
CS Unite Genet Mol Bacterienne, Inst Pasteur, 28 Rue Dr Roux, F-75724, Paris, 15, France
demangel@pasteur.fr
SO Infection and Immunity, (April 2004) Vol. 72, No. 4, pp. 2170-2176. print. ISSN: 0019-9567 (ISSN print).
DT Article
LA English
ED Entered STN: 17 Nov 2004
Last Updated on STN: 17 Nov 2004
AB The protective efficacy of Mycobacterium bovis BCG can be markedly augmented by stable integration of Mycobacterium tuberculosis genomic region RD1. BCG complemented with RD1 (BCG ::RDI) encodes nine additional proteins. Among them, 10-kDa culture

filtrate protein (CFP-10) and ESAT-6 (6-kDa early secreted antigenic target) are low-molecular-weight proteins that induce potent Th1 responses. Using pools of synthetic peptides, we have examined the potential immunogenicity of four other RD1 products (PE35, PPE68, Rv3878, and Rv3879c). PPE68, the protein encoded by rv3873, was the only one to elicit gamma interferon (IFN-gamma)-producing cells in C57BL/6 mice infected with M. tuberculosis. Anti-PPE68 T cells were predominantly raised against an epitope mapped in the N-terminal end of the protein. Importantly, inactivation of rv3873 in BCG::RD1 did not modify CFP-10 and ESAT-6 secretion. Moreover, the generation of IFN-gamma responses to these antigens following immunization with BCG::RD1 was independent of PPE68 expression. Taken together, these results show that PPE68 is an immunogenic product of the RD1 region, which does not interfere with the secretion and immunogenicity of CFP-10 and ESAT-6.

L12 ANSWER 9 OF 15 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 8
AN 2003:249243 BIOSIS
DN PREV200300249243
TI Recombinant BCG exporting ESAT-6 confers enhanced protection against tuberculosis.
AU Pym, Alexander S.; Brodin, Priscille; Majlessi, Laleh; Brosch, Roland; Demangel, Caroline; Williams, Ann; Griffiths, Karen E.; Marchal, Gilles; Leclerc, Claude; Cole, Stewart T. [Reprint Author]
CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, Paris, France
stcole@pasteur.fr
SO Nature Medicine, (May 2003) Vol. 9, No. 5, pp. 533-539. print.
ISSN: 1078-8956 (ISSN print).
DT Article
LA English
ED Entered STN: 28 May 2003
Last Updated on STN: 28 May 2003
AB The live tuberculosis vaccines Mycobacterium bovis BCG (bacille Calmette-Guerin) and Mycobacterium microti both lack the potent, secreted T-cell antigens ESAT-6 (6-kDa early secretory antigenic target) and CFP-10 (10-kDa culture filtrate protein). This is a result of independent deletions in the region of deletion-1 (RD1) locus, which is intact in virulent members of the Mycobacterium tuberculosis complex. To increase their immunogenicity and protective capacity, we complemented both vaccines with different constructs containing the esxA and esxB genes, which encode ESAT-6 and CFP-10 respectively, as well as a variable number of flanking genes. Only reintroduction of the complete locus, comprising at least 11 genes, led to full secretion of the antigens and resulted in specific ESAT-6-dependent immune responses; this suggests that the flanking genes encode a secretory apparatus. Mice and guinea pigs vaccinated with the recombinant strain BCG::RD1-2F9 were better protected against challenge with M. tuberculosis, showing less severe pathology and reduced dissemination of the pathogen, as compared with control animals immunized with BCG alone.

L12 ANSWER 10 OF 15 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 9
AN 2002:273545 BIOSIS
DN PREV200200273545
TI Autocrine IL-10 impairs dendritic cell (DC)-derived immune responses to mycobacterial infection by suppressing DC trafficking to draining lymph nodes and local IL-12 production.
AU Demangel, Caroline; Bertolino, Patrick; Britton, Warwick J. [Reprint author]
CS Centenary Institute of Cancer Medicine and Cell Biology, Newtown, NSW, 2042, Australia
wbritton@medicine.usyd.edu.au
SO European Journal of Immunology, (April, 2002) Vol. 32, No. 4, pp.

994-1002. print.
CODEN: EJIMAF. ISSN: 0014-2980.
DT Article
LA English
ED Entered STN: 8 May 2002
Last Updated on STN: 8 May 2002
AB The production of IL-12 by dendritic cells (DC) early in an immune response is considered critical for the polarization of CD4+ T lymphocyte response towards a Th1 pattern, a key process in the clearance of intracellular pathogens. Infection of bone marrow-derived DC with Mycobacterium bovis Bacillus Calmette Guerin (BCG) induced a concurrent and dose-dependent release of IL-10 and IL-12. Here we examined whether the production of IL-10 by DC affected their IL-12 response to mycobacterial infection and the generation of protective immune responses in vivo. Compared to wild-type (WT) DC, DC deficient for IL-10 synthesis (IL-10-/-) showed increased IL-12 production in response to BCG infection and CD40 stimuli in vitro. Moreover, when transferred into mice, infected IL-10-/- DC were more efficient than WT DC at inducing IFN-gamma production to mycobacterial antigens in the draining lymph nodes (DLN). This effect was associated with increased trafficking of IL-10-/- DC to the DLN and enhanced IL-12 production by DC within the DLN. These data show that autocrine IL-10 exerts a dual inhibitory effect on the induction of primary immune responses by DC: first, by down-regulating the migration of infected DC to the DLN and second, by modulating the IL-12 production by DC in the DLN.

L12 ANSWER 11 OF 15 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 10
AN 2001:345375 BIOSIS
DN PREV200100345375
TI Priming by DNA immunization augments protective efficacy of Mycobacterium bovis bacille Calmette-Guerin against tuberculosis.
AU Feng, Carl G.; Palendira, Umaimainthan; Demangel, Caroline; Spratt, Joanne M.; Malin, Adam S.; Britton, Warwick J. [Reprint author]
CS Centenary Institute of Cancer Medicine and Cell Biology, Newtown, NSW, 2042, Australia
wbritton@medicine.usyd.edu.au
SO Infection and Immunity, (June, 2001) Vol. 69, No. 6, pp. 4174-4176. print.
CODEN: INFIBR. ISSN: 0019-9567.
DT Article
LA English
ED Entered STN: 25 Jul 2001
Last Updated on STN: 19 Feb 2002
AB Sequential immunization with mycobacterial antigen Ag85B-expressing DNA and Mycobacterium bovis bacille Calmette-Guerin (BCG) was more effective than BCG immunization in protecting against Mycobacterium tuberculosis infection. Depletion of the CD8+ T cells in the immunized mice impaired protection in their spleens, indicating that this improved efficacy was partially mediated by CD8+ T cells.

L12 ANSWER 12 OF 15 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 11
AN 2001:211937 BIOSIS
DN PREV200100211937
TI Stimulation of dendritic cells via CD40 enhances immune responses to Mycobacterium tuberculosis infection.
AU Demangel, Caroline; Palendira, Umaimainthan; Feng, Carl G.; Heath, Andrew W.; Bean, Andrew G. D.; Britton, Warwick J. [Reprint author]
CS Centenary Institute of Cancer Medicine and Cell Biology, Newtown, NSW, 2042, Australia
wbritton@medicine.usyd.edu.au
SO Infection and Immunity, (April, 2001) Vol. 69, No. 4, pp. 2456-2461. print.
CODEN: INFIBR. ISSN: 0019-9567.

DT Article
LA English
ED Entered STN: 2 May 2001
Last Updated on STN: 18 Feb 2002
AB The resolution of pulmonary tuberculosis (TB) critically depends on the development of the Th1 type of immune responses, as exemplified by the exacerbation of TB in IL-12-deficient mice. Therefore, vaccination strategies optimizing IL-12 production by antigen-presenting cells (APC) in response to mycobacteria may have enhanced protective efficacy. Since dendritic cells (DC) are the critical APC for activation of CD4+ and CD8+ T cells, we examined whether stimulation of Mycobacterium bovis bacillus Calmette Guerin (BCG)-infected DC via CD40 increased their ability to generate Th1-oriented cellular immune responses. Incubation of DC with an agonistic anti-CD40 antibody activated CD40 signaling in DC, as shown by increased expression of major histocompatibility complex class II and costimulatory molecules, mRNA production for proinflammatory cytokines and interleukin 12 (IL-12) p40. This activation pattern was maintained when DC were stimulated with anti-CD40 antibody and infected with BCG. Importantly, CD40-stimulated BCG-infected DC displayed increased capacity to release bioactive IL-12 and to activate gamma interferon (IFN-gamma) producing T cells in vitro. Moreover, when C57BL/6 mice were immunized with these DC and challenged with aerosol Mycobacterium tuberculosis, increased levels of mRNA for IL-12 p40, IL-18, and IFN-gamma were present in the draining mediastinal lymph nodes. However, the mycobacterial burden in the lungs was not reduced compared to that in mice immunized with BCG-infected non-CD40-stimulated DC. Therefore, although the manipulation of DC via CD40 is effective for enhancing immune responses to mycobacteria in vivo, additional strategies are required to increase protection against virulent M. tuberculosis infection.

L12 ANSWER 13 OF 15 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 12
AN 2001:258293 BIOSIS
DN PREV200100258293
TI Dendritic cells infected with Mycobacterium bovis bacillus Calmette Guerin activate CD8+ T cells with specificity for a novel mycobacterial epitope.
AU Feng, Carl G.; Demangel, Caroline; Kamath, Arun T.; Macdonald, Murdo; Britton, Warwick J. [Reprint author]
CS Centenary Institute of Cancer Medicine and Cell Biology, Newtown, NSW, 2042, Australia
SO International Immunology, (April, 2001) Vol. 13, No. 4, pp. 451-458. print.
ISSN: 0953-8178.
DT Article
LA English
ED Entered STN: 30 May 2001
Last Updated on STN: 19 Feb 2002
AB Although CD4+ T cells are essential for protective immunity against Mycobacterium tuberculosis infection, recent reports indicate that CD8+ T cells may also play a critical role in the control of this infection. However, the epitope specificity and the mechanisms of activation of mycobacteria-reactive CD8+ T cells are poorly characterized. In order to study the CD8+ T cell responses to the model mycobacterial antigen, MPT64, we used recombinant vaccinia virus expressing MPT64 (VVWR-64) and a panel of MPT64-derived peptides to establish that the peptide MPT64190-198 contains an H-2Db-restricted CD8+ T cell epitope. A cytotoxic T lymphocyte response to this peptide could be demonstrated in M. bovis bacillus Calmette Guerin (BCG)-infected mice following repeated in vitro stimulation. When bone marrow-derived dendritic cells (DC) were infected with BCG, the expression of MHC class I molecules by DC was up-regulated in parallel with MHC class II and B7-2, whereas CD1d expression level was not modified. Moreover, BCG-infected DC activated MPT64190-198-specific CD8+ T cells to secrete IFN-gamma,

although with a lower efficacy than VVWR-64-infected DC. The production of IFN-gamma by MPT64190-198-specific CD8+ T cells was inhibited by antibodies to MHC class I, but not to CD1d. These data suggest that mycobacteria-specific CD8+ T cells are primed during infection. Therefore, anti-mycobacterial vaccine strategies targeting the activation of specific CD8+ T cells by DC may have improved protective efficacy.

L12 ANSWER 14 OF 15 MEDLINE on STN
AN 2000436797 MEDLINE
DN PubMed ID: 10947855
TI Interaction of dendritic cells with mycobacteria: where the action starts.
AU Demangel C; Britton W J
CS Centenary Institute of Cancer Medicine and Cell Biology, University of Sydney, Department of Medicine, Sydney, New South Wales, Australia.
SO Immunology and cell biology, (2000 Aug) Vol. 78, No. 4, pp. 318-24. Ref: 69
Journal code: 8706300. ISSN: 0818-9641.
CY Australia
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
LA English
FS Priority Journals; AIDS
EM 200009
ED Entered STN: 28 Sep 2000
Last Updated on STN: 28 Sep 2000
Entered Medline: 15 Sep 2000
AB Dendritic cells (DC) are the major antigen-presenting cells in the induction of cellular responses to intracellular pathogens, such as mycobacteria. Recent studies have shown that they also play a critical role in the regulation of immune responses. The interaction of DC with microbial antigens may be the controlling factor in the development of a Th1-orientated protective immunity. Analysis of the innate response of DC to mycobacteria and the involvement of the DC receptors in antigen recognition have highlighted the pivotal role of these cells in T-cell activation. Mycobacteria-infected DC have an enhanced capacity to release pro-inflammatory cytokines and chemokines and are potent inducers of interferon-gamma-producing cells in vivo. Therefore, DC manipulation for maximal antigen presentation and Th1 cytokine production may form the basis of a new generation of vaccines, with improved efficacy against mycobacterial infections.

L12 ANSWER 15 OF 15 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 13
AN 1999:311705 BIOSIS
DN PREV199900311705
TI Protection against aerosol Mycobacterium tuberculosis infection using Mycobacterium bovis Bacillus Calmette Guerin-infected dendritic cells.
AU Demangel, Caroline; Bean, Andrew G. D.; Martin, Ela; Feng, Carl G.; Kamath, Arun T.; Britton, Warwick J. [Reprint author]
CS Centenary Institute of Cancer Medicine and Cell Biology, Newtown, NSW, 2042, Australia
SO European Journal of Immunology, (June, 1999) Vol. 29, No. 6, pp. 1972-1979. print.
CODEN: EJIMAF. ISSN: 0014-2980.
DT Article
LA English
ED Entered STN: 17 Aug 1999
Last Updated on STN: 17 Aug 1999
AB In the lung, dendritic cells (DC) are key antigen-presenting cells capable of triggering specific cellular responses to inhaled pathogens, and thus, they may be important in the initiation of an early response to mycobacterial infections. The ability of DC to enhance antigen presentation to naive T cells within the lungs was characterized with respect to Mycobacterium bovis Bacillus Calmette Guerin (BCG)

vaccination against M. tuberculosis infection. In vitro derived DC were infected with BCG, which induced their maturation, as shown by the increased expression of MHC class II antigens, CD80 and CD86 co-stimulatory molecules. The synthesis of mRNA for IL-1, IL-6, IL-12, IL-10 and IL-1 receptor antagonist was also enhanced. When administered intratracheally in mice, infected DC induced a potent T cell response and the production of IFN-gamma to mycobacterial antigens in the mediastinal lymph nodes, leading to a significant protection against aerosol M. tuberculosis infection. Intriguingly, although the vaccination schedule for BCG-infected DC was much shorter than subcutaneous BCG vaccination (7 days as compared to 100 days), both types of vaccination showed similar levels of protection. These data confirm that DC can be potent inducers of a cellular immune response against mycobacteria and support the concept of combining DC strategies with mycobacterial vaccines for protective immunity against tuberculosis.

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E2            2     LECLERC CLAUD/AU
E3          350 --> LECLERC CLAUDE/AU
E4            8     LECLERC CLAUDE D/AU
E5            2     LECLERC COORNAERT L/AU
E6            5     LECLERC COREY/AU
E7            3     LECLERC COREY A/AU
E8            1     LECLERC COREY ANDREW/AU
E9            2     LECLERC CYNTHIA/AU
E10           7     LECLERC CYNTHIA L/AU
E11         266     LECLERC D/AU
E12           1     LECLERC D B/AU

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               D"/AU) AND (BCG OR MICROTI)

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PROCESSING COMPLETED FOR L13
L14           24 DUP REM L13 (36 DUPLICATES REMOVED)

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YOU HAVE REQUESTED DATA FROM 24 ANSWERS - CONTINUE? Y/(N):y

L14  ANSWER 1 OF 24  CAPLUS  COPYRIGHT 2007 ACS on STN
AN   2007:41383  CAPLUS
DN   146:140994
TI   Modified ESAT-6 derived from Mycobacterium tuberculosis and Mycobacterium
     leprae as vaccines for inducing interferon γ response to ESAT-6
     and/or CFP-10 against infection
IN   Brosch, Roland; Brodin, Priscille; Cole, Stewart; Majlessi, Laleh;
     Leclerc, Claude
PA   Fr.
SO   U.S. Pat. Appl. Publ., 64pp.
     CODEN: USXXCO
DT   Patent
LA   English
FAN.CNT 1
     PATENT NO.          KIND   DATE        APPLICATION NO.         DATE
     --------------      ----   --------    --------------------    --------
PI   US 2007009547        A1    20070111    US 2006-455929          20060620
     WO 2007010413        A2    20070125    WO 2006-IB2884          20060622
     WO 2007010413        A3    20070830
         W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH,
             CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD,
             GE, GH, GM, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP,
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KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW
RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AP, EA, EP, OA
PRAI US 2005-692561P P 20050622
AB A genetically modified strain of M. tuberculosis or Mycobacterium bovis BCG is provided, wherein the genetically modified strain comprises at least one modified sequence comprising SEQ ID NO: 1, SEQ ID NO: 2, or both, having at least one mutation at T2, Q4, F8, A14, L28, L29, W43, G45, Q55, Q56, N66, M83, V90, M93, or F94. In a preferred embodiment, the mutation is at least one of T2H, Q4L, F8I, A14R, L28A, L29S, W43R, G45T, Y51, Q55I, Q56A, N66I, N66A, M83I, V90R, M93T, or F94Q. Similarly, the genetically modified strain may also secrete ESAT-6 with a a histidine tag, tetra-cysteine tag or FLAG-tag, a GFP-fusion, or a short truncation at the C-terminal end of less than 20 amino acids.

L14 ANSWER 2 OF 24 CAPLUS COPYRIGHT 2007 ACS on STN
AN 2007:788499 CAPLUS
DN 147:164307
TI Inhibition of phagosome maturation by mycobacteria does not interfere with presentation of mycobacterial antigens by MHC molecules
AU Majlessi, Laleh; Combaluzier, Benoit; Albrecht, Imke; Garcia, Jessica E.; Nouze, Clemence; Pieters, Jean; Leclerc, Claude
CS Unite de Regulation Immunitaire et Vaccinologie, Institut Pasteur, Paris, Fr.
SO Journal of Immunology (2007), 179(3), 1825-1833
CODEN: JOIMA3; ISSN: 0022-1767
PB American Association of Immunologists
DT Journal
LA English
AB Pathogenic mycobacteria escape host innate immune responses by surviving within phagosomes of host macrophages and blocking their delivery to lysosomes. Avoiding lysosomal delivery may also be involved in the capacity of living mycobacteria to modulate MHC class I- or II-dependent T cell responses, which may contribute to their pathogenicity in vivo. In this study, the authors show that the presentation of mycobacterial Ags is independent of the site of intracellular residence inside professional APCs. Infection of mouse macrophages or dendritic cells in vitro with mycobacterial mutants that are unable to escape lysosomal transfer resulted in an identical efficiency of Ag presentation compared with wild-type mycobacteria. Moreover, in vivo, such mutants induced CD4+ Th1 or CD8+ CTL responses in mice against various mycobacterial Ags that were comparable to those induced by their wild-type counterparts. These results suggest that the limiting factor for the generation of an adaptive immune response against mycobacteria is not the degree of lysosomal delivery. These findings are important in the rational design of improved vaccines to combat mycobacterial diseases.
RE.CNT 57 THERE ARE 57 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 3 OF 24 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 1
AN 2006:351223 BIOSIS
DN PREV200600347024
TI High frequency of CD4(+) T cells specific for the TB10.4 protein correlates with protection against Mycobacterium tuberculosis infection.
AU Hervas-Stubbs, Sandra; Majlessi, Laleh; Simsova, Marcela; Morova, Jana; Rojas, Marie-Jesus; Nouze, Clemence; Brodin, Priscille; Sebo, Peter; Leclerc, Claude [Reprint Author]

CS Inst Pasteur, INSERM, E352, 25 Rue Docteur Roux, F-75724 Paris 15, France
cleclerc@pasteur.fr
SO Infection and Immunity, (JUN 2006) Vol. 74, No. 6, pp. 3396-3407.
CODEN: INFIBR. ISSN: 0019-9567.
DT Article
LA English
ED Entered STN: 12 Jul 2006
Last Updated on STN: 12 Jul 2006
AB TB10.4 is a newly identified antigen of Mycobacterium tuberculosis recognized by human and murine T cells upon mycobacterial infection. Here, we show that immunization with Mycobacterium bovis BCG induces a strong, genetically controlled, Th1 immune response against TB10.4 in mice. BALB/c and C57BL/6 strains behave as high and low responders to TB10.4 protein, respectively. The TB10.4:74-88 peptide was identified as an immunodominant CD4(+) T-cell epitope for H-2(d) mice. Since recent results, as well as the present study, have raised interest in TB10.4 as a subunit vaccine, we analyzed immune responses induced by this antigen delivered by a new vector, the adenylate cyclase (CyaA) of Bordetella pertussis. CyaA is able to target dendritic cells and to deliver CD4(+) or CD8(+) T-cell epitopes to the major histocompatibility complex class II/I molecule presentation pathways, triggering specific Th1 or cytotoxic T-lymphocyte (CTL) responses. Several CyaA harboring either the entire TB10.4 protein or various subfragments containing the TB10.4:20-28 CTL epitope were shown to induce TB10.4-specific Th1 CD4(+) and CD8(+) T-cell responses. However, none of the recombinant CyaA, injected in the absence of adjuvant, was able to induce protection against M. tuberculosis infection. In contrast, TB10.4 protein administered with a cocktail of strong adjuvants that triggered a strong Th1 CD4(+) T-cell response induced significant protection against M. tuberculosis challenge. These results confirm the potential value of the TB10.4 protein as a candidate vaccine and show that the presence of high frequencies of CD4(+) T cells specific to this strong immunogen correlates with protection against M. tuberculosis infection.

L14 ANSWER 4 OF 24 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 2
AN 2006:370142 BIOSIS
DN PREV200600369174
TI An increase in antimycobacterial Th1-cell responses by prime-boost protocols of immunization does not enhance protection against tuberculosis.
AU Majlessi, Laleh [Reprint Author]; Simsova, Marcela; Jarvis, Zdenka; Brodin, Priscille; Rojas, Marie-Jesus; Bauche, Cecile; Nouze, Clemence; Ladant, Daniel; Cole, Stewart T.; Sebo, Peter; Leclerc, Claude
CS Inst Pasteur, Unite Biol Regulat Immunitaries, INSERM, 25 Rue Dr Roux,E 352, F-75724 Paris 15, France
lmajless@pasteur.fr
SO Infection and Immunity, (APR 2006) Vol. 74, No. 4, pp. 2128-2137.
CODEN: INFIBR. ISSN: 0019-9567.
DT Article
LA English
ED Entered STN: 26 Jul 2006
Last Updated on STN: 26 Jul 2006
AB Bordetella pertussis adenylate cyclase (CyaA) toxoid is a powerful nonreplicative immunization vector targeting dendritic cells, which has already been used successfully in prophylactic and therapeutic vaccination in various preclinical animal models. Here, we investigated the potential of CyaA, harboring strong mycobacterial immunogens, i.e., the immunodominant regions of antigen 85A or the complete sequence of the 6-kDa early secreted antigenic target (ESAT-6) protein, to induce anti mycobacterial immunity. By generating T-cell hybridomas or by using T cells from mice infected with mycobacteria, we first demonstrated that the in vitro delivery of 85A or ESAT-6 to antigen-presenting cells by CyaA leads to processing and presentation, by major histocompatibility complex

class II molecules, of the same epitopes as those displayed upon mycobacterial infection. Importantly, compared to the recombinant protein alone, the presentation of ESAT-6 in vitro was 100 times more efficient upon its delivery to antigen-presenting cells in fusion to CyaA. Immunization with CyaA-85A or CyaA-ESAT-6 in the absence of any adjuvant induced strong antigen-specific lymphoproliferative, interleukin-2 (IL-2) and gamma interferon (IFN-gamma) cytokine responses, in the absence of any IL-4 or IL-5 production. When used as boosters after priming with a BCG expressing ESAT-6, the CyaA-85A and CyaA-ESAT-6 proteins were able to strikingly increase the sensitivity and intensity of proliferative and Th1-polarized responses and notably the frequency of antigen-specific IFN-gamma-producing CD4(+) T cells. However, immunization with these CyaA constructs as subunit vaccines alone or as boosters did not allow induction or improvement of protection against Mycobacterium tuberculosis infection. These results question the broadly admitted correlation between the frequency of IFN-gamma-producing CD4(+) T cells and the level of protection against tuberculosis.

L14 ANSWER 5 OF 24 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 3
AN 2006:176683 BIOSIS
DN PREV200600166449
TI Dissection of ESAT-6 system 1 of Mycobacterium tuberculosis and impact on immunogenicity and virulence.
AU Brodin, Priscille; Majlessi, Laleh; Marsollier, Laurent; de Jonge, Marien I.; Bottai, Daria; Demangel, Caroline; Hinds, Jason; Neyrolles, Olivier; Butcher, Philip D.; Leclerc, Claude; Cole, Stewart T.; Brosch, Roland [Reprint Author]
CS Inst Pasteur, Unite Genet Mol Bacterienne, 25-28 Rue Docteur Roux, F-75724 Paris 15, France
rbrosch@pasteur.fr
SO Infection and Immunity, (JAN 2006) Vol. 74, No. 1, pp. 88-98.
CODEN: INFIBR. ISSN: 0019-9567.
DT Article
LA English
ED Entered STN: 9 Mar 2006
Last Updated on STN: 9 Mar 2006
AB The dedicated secretion system ESX-1 of Mycobacterium tuberculosis encoded by the extended RD1 region (extRD1) assures export of the ESAT-6 protein and its partner, the 10-kDa culture filtrate protein CFP-10, and is missing from the vaccine strains M. bovis BCG and M. microti. Here, we systematically investigated the involvement of each individual ESX-1 gene in the secretion of both antigens, specific immunogenicity, and virulence. ESX-1-complemented BCG and M. microti strains were more efficiently engulfed by bone-marrow-derived macrophages than controls, and this may account for the enhanced in vivo growth of ESX-1-carrying strains. Inactivation of gene pe35 (Rv3872) impaired expression of CFP-10 and ESAT-6, suggesting a role in regulation. Genes Rv3868, Rv3869, Rv3870, Rv3871, and Rv3877 encoding an ATP-dependent chaperone and translocon were essential for secretion of ESAT-6 and CFP-10 in contrast to ppe68 Rv3873 and Rv3876, whose inactivation did not impair secretion of ESAT-6. A strict correlation was found between ESAT-6 export and the generation of ESAT-6 specific T-cell responses in mice. Furthermore, ESAT-6 secretion and specific immunogenicity were almost always correlated with enhanced virulence in the SCID mouse model. Only loss of Rv3865 and part of Rv3866 did not affect ESAT-6 secretion or immunogenicity but led to attenuation. This suggests that Rv3865/66 represent a new virulence factor that is independent from ESAT-6 secretion. The present study has allowed us to identify new aspects of the extRD1 region of M. tuberculosis and to explore its role in the pathogenesis of tuberculosis.

L14 ANSWER 6 OF 24 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 4

AN 2006:7638 BIOSIS
DN PREV200600007409
TI Functional analysis of early secreted antigenic target-6, the dominant T-cell antigen of Mycobacterium tuberculosis, reveals key residues involved in secretion, complex formation, virulence, and immunogenicity.
AU Brodin, Priscille; de Jonge, Marien I.; Majlessi, Laleh; Leclerc, Claude; Nilges, Michael; Cole, Stewart T.; Brosch, Roland [Reprint Author]
CS Inst Pasteur, Unite Genet Mol Bacterienne, 28 Rue Dr Roux, F-75724 Paris, France
rbrosch@pasteur.fr
SO Journal of Biological Chemistry, (OCT 7 2005) Vol. 280, No. 40, pp. 33953-33959.
CODEN: JBCHA3. ISSN: 0021-9258.
DT Article
LA English
ED Entered STN: 14 Dec 2005
Last Updated on STN: 14 Dec 2005
AB Proteins of the 6-kDa early secreted antigenic target (ESAT-6) secretion system-1 of Mycobacterium tuberculosis are not only strongly involved in the anti-mycobacterial Th1-host immune response but are also key players for virulence. In this study, protein engineering together with bioinformatic, immunological, and virulence analyses allowed us to pinpoint regions of the ESAT-6 molecule that are critical for its biological activity in M. tuberculosis. Mutation of the Trp-Xaa-Gly motif, conserved in a wide variety of ESAT-6-like proteins, abolished complex formation with the partner protein CFP-10, induction of specific T-cell responses, and virulence. Replacement of conserved Leu residues interfered with secretion, coiled-coil formation, and virulence, whereas certain mutations at the extreme C terminus did not affect secretion but caused attenuation, possibly because of altered ESAT-6 targeting or trafficking. In contrast, the mutation of several residues on the outer surface of the four-helical bundle structure of the ESAT-6 center dot CFP-10 complex showed much less effect. Construction of recombinant BCG expressing ESAT-6 with a C-terminal hexahistidine tag allowed us to co-purify ESAT-6 and CFP-10, experimentally confirming their strong interaction both in and outside of the mycobacterial cell. The strain induced potent, antigen-specific T-cell responses and intermediate in vivo growth in mice, suggesting that it remained immunogenic and biologically active despite the tag. Together with previous NMR data, the results of this study have allowed a biologically relevant model of the ESAT-6 center dot CFP-10 complex to be constructed that is critical for understanding the structure-function relationship in tuberculosis pathogenesis.

L14 ANSWER 7 OF 24 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 5
AN 2005:215081 BIOSIS
DN PREV200510005668
TI Influence of ESAT-6 secretion system 1 (RD1) of Mycobacterium tuberculosis on the interaction between mycobacteria and the host immune system.
AU Majlessi, Laleh [Reprint Author]; Brodin, Priscille; Brosch, Roland; Rojas, Marie-Jesus; Khun, Huot; Huerre, Michel; Cole, Stewart T.; Leclerc, Claude
CS Inst Pasteur, Unite Biol Regulat Immun, INSERM, Equipe 352, 25,Rue Dr Roux, F-75724 Paris 15, France
lmajless@pasteur.fr
SO Journal of Immunology, (MAR 15 2005) Vol. 174, No. 6, pp. 3570-3579.
CODEN: JOIMA3. ISSN: 0022-1767.
DT Article
LA English
ED Entered STN: 10 Jun 2005
Last Updated on STN: 10 Jun 2005
AB The chromosomal locus encoding the early secreted antigenic target, 6 kDa (ESAT-6) secretion system I of Mycobacterium tuberculosis, also referred

to as "region of difference I (RD1)," is absent from Mycobacterium bovis bacillus Calmette-Guerin (BCG). In this study, using low-dose aerosol infection in mice, we demonstrate that BCG complemented with RD1 (BCG::RD1) displays markedly increased virulence which albeit does not attain that of M. tuberculosis H37Rv. Nevertheless, phenotypic and functional analyses of immune cells at the site of infection show that the capacity of BCG::RD1 to initiate recruitment/activation of immune cells is compaiable to that of fully virulent H37Rv. Indeed, in contrast to the parental BCG, BCG::RDI mimics H37Rv and induces substantial influx of activated (CD44(high)CD45RB(-)CD62L(-)) or effector (CD45RB(-)CD27(-)) T cells and of activated CD11c(+)CD11b(high) cells to the lungs of aerosol-infected mice. For the first time, using in vivo analysis of transcriptome of inflammatory cytokines and chemokines of lung interstitial CD11c(+) cells, we show that in a low-dose aerosol infection model, BCG::RDI triggered an activation/inflammation program comparable to that induced by H37Rv while parental BCG, due to its overattenuation, did not initiate the activation program in lung interstitial CD11c(+) cells. Thus, products encoded by the ESAT-6 secretion system 1 of M. tuberculosis profoundly modify the interaction between mycobacteria and the host innate and adaptive immune system. These modifications can explain the previously described improved protective capacity of BCG::RDI vaccine candidate against M. tuberculosis challenge. The Journal of Immunology, 2005, 174: 3570-3579.

L14 ANSWER 8 OF 24 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 6
AN 2005:278319 BIOSIS
DN PREV200510068982
TI Efficient ex vivo stimulation of Mycobacterium tuberculosis-specific T cells by genetically detoxified Bordetella pertussis adenylate cyclase antigen toxoids.
AU Wilkinson, Katalin A.; Simsova, Marcela; Scholvinck, Elisabeth; Sebo, Peter; Leclerc, Claude; Vordermeier, H. Martin; Dickson, Stuart J.; Brown, Jillian R.; Davidson, Robert N.; Pasvol, Geoffrey; Levin, Michael; Wilkinson, Robert J. [Reprint Author]
CS Univ London Imperial Coll Sci Technol and Med, Wright Fleming Inst, Wellcome Trust Ctr Res Clin Trop Med, Div Med, Norfolk Pl, London W2 1PG, UK
r.j.wilkinson@imperial.ac.uk
SO Infection and Immunity, (MAY 2005) Vol. 73, No. 5, pp. 2991-2998. CODEN: INFIBR. ISSN: 0019-9567.
DT Article
LA English
ED Entered STN: 27 Jul 2005
Last Updated on STN: 27 Jul 2005
AB Mycobacterium tuberculosis is a significant threat to global health. Mycobacterium bovis BCG vaccine provides only partial protection, and the skin test reagent used to aid diagnosis of both active and latent tuberculosis, purified protein derivative (PPD), lacks specificity and sensitivity. The use of genetically detoxified Bordetella pertussis adenylate cyclase toxin (CyaA) as a delivery system for two immunodominant proteins of M. tuberculosis that are of greater specificity than PPD, early-secreted antigenic target 6-kDa protein (ESAT-6) and culture filtrate protein 10 (CFP-10), was therefore investigated. CyaA toxoids incorporating these antigens were able to restimulate T cells from more than 91% tuberculosis patients and healthy sensitized donors. Delivery of antigen by CyaA decreased by 10-fold the amount of ESAT-6 and CFP-10 required to restimulate T cells, and in low responders, the overall frequency of gamma interferon-producing cells detected by enzyme-linked immunospot assay was increased ($P < 0.01$ for both antigens). Delivery of ESAT-6 and CFP-10 by CyaA enabled the detection of both CD4(+) and CD8(+) T cells: these responses could be blocked by inhibition of major histocompatibility complex class II or class I, respectively. Covalent

linkage of antigen to the CyaA vector was required for enhancement to occur, as a mixture of mock CyaA toxoid plus recombinant ESAT-6 did not lead to enhancement. In a simplified whole-blood model to detect tuberculosis infection, the frequency of positive responses to CFP-10 was increased by CyaA delivery, a potentially important attribute that could facilitate the identification of latent infection.

L14 ANSWER 9 OF 24 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 7
AN 2004:438631 BIOSIS
DN PREV200400437455
TI Cell envelope protein PPE68 contributes to Mycobacterium tuberculosis RDI immunogenicity independently of a 10-kilodalton culture filtrate protein and ESAT-6.
AU Demangel, Caroline [Reprint Author]; Brodin, Priscille; Cockle, Paul J.; Brosch, Roland; Majlessi, Laleh; Leclerc, Claude; Cole, Stewart T.
CS Unite Genet Mol Bacterienne, Inst Pasteur, 28 Rue Dr Roux, F-75724, Paris, 15, France
demangel@pasteur.fr
SO Infection and Immunity, (April 2004) Vol. 72, No. 4, pp. 2170-2176. print.
ISSN: 0019-9567 (ISSN print).
DT Article
LA English
ED Entered STN: 17 Nov 2004
Last Updated on STN: 17 Nov 2004
AB The protective efficacy of Mycobacterium bovis BCG can be markedly augmented by stable integration of Mycobacterium tuberculosis genomic region RD1. BCG complemented with RD1 (BCG ::RDI) encodes nine additional proteins. Among them, 10-kDa culture filtrate protein (CFP-10) and ESAT-6 (6-kDa early secreted antigenic target) are low-molecular-weight proteins that induce potent Th1 responses. Using pools of synthetic peptides, we have examined the potential immunogenicity of four other RD1 products (PE35, PPE68, Rv3878, and Rv3879c). PPE68, the protein encoded by rv3873, was the only one to elicit gamma interferon (IFN-gamma)-producing cells in C57BL/6 mice infected with M. tuberculosis. Anti-PPE68 T cells were predominantly raised against an epitope mapped in the N-terminal end of the protein. Importantly, inactivation of rv3873 in BCG::RD1 did not modify CFP-10 and ESAT-6 secretion. Moreover, the generation of IFN-gamma responses to these antigens following immunization with BCG::RD1 was independent of PPE68 expression. Taken together, these results show that PPE68 is an immunogenic product of the RD1 region, which does not interfere with the secretion and immunogenicity of CFP-10 and ESAT-6.

L14 ANSWER 10 OF 24 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 8
AN 2004:326990 BIOSIS
DN PREV200400328635
TI Enhanced protection against tuberculosis by vaccination with recombinant Mycobacterium microti vaccine that induces T cell immunity against region of difference 1 antigens.
AU Brodin, Priscille; Majlessi, Laleh; Brosch, Roland; Smith, Debbie; Bancroft, Gregory; Clark, Simon; Williams, Ann; Leclerc, Claude; Cole, Stewart T. [Reprint Author]
CS Unite Genet Mol Bacterienne, Inst Pasteur, 28 Rue Docteur Roux, F-75724, Paris, 15, France
stcole@pasteur.fr
SO Journal of Infectious Diseases, (July 1 2004) Vol. 190, No. 1, pp. 115-122. print.
CODEN: JIDIAQ. ISSN: 0022-1899.
DT Article
LA English
ED Entered STN: 29 Jul 2004

Last Updated on STN: 29 Jul 2004

AB Mycobacterium microti, the vole bacillus, which was used as a live vaccine against tuberculosis until the 1970s, confers the same protection in humans as does Mycobacterium bovis bacille Calmette-Guerin (BCG). However, because the efficacy of the BCG vaccine varies considerably, we have tried to develop a better vaccine by reintroducing into M. microti the complete region of difference 1 (RD1), which is required for secretion of the potent T cell antigens early secreted antigen target (ESAT)-6 and culture filtrate protein (CFP)-10. The resultant recombinant strain, M. microti OV254::RD1-2F9, induced specific ESAT-6 and CFP-10 immune responses in mice with CD8+ T lymphocytes that had strong expression of the CD44hi activation marker. This vaccine also displayed better efficacy against disseminated disease in the mouse and the guinea pig models of tuberculosis than was seen in animals vaccinated with M. microti alone or with BCG. The M. microti OV254::RD1-2F9 vaccine was less virulent and persistent in mice and than was BCG ::RD1-2F9 may represent a safer alternative to BCG::RD1-2F9.

L14 ANSWER 11 OF 24 CAPLUS COPYRIGHT 2007 ACS on STN
AN 2003:791413 CAPLUS
DN 139:304478
TI Identification of virulence associated regions RD1 and RD5 enabling the development of improved vaccines of M. bovis BCG and M. microti
IN Cole, Stewart; Pym, Alexander S.; Brosch, Roland; Brodin, Priscille; Majlessi, Laleh; Leclerc, Claude
PA Institut Pasteur, Fr.
SO Eur. Pat. Appl., 58 pp.
CODEN: EPXXDW
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 1350839	A1	20031008	EP 2002-290864	20020405
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
	CA 2481318	A1	20031016	CA 2003-2481318	20030401
	WO 2003085098	A2	20031016	WO 2003-IB1789	20030401
	WO 2003085098	A3	20040129		
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
	AU 2003223039	A1	20031020	AU 2003-223039	20030401
	EP 1492867	A2	20050105	EP 2003-719008	20030401
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
	US 2005220811	A1	20051006	US 2004-510021	20041001
PRAI	EP 2002-290864	A	20020405		
	WO 2003-IB1789	W	20030401		

AB The present invention relates to a strain of Mycobacterium bovis BCG or Mycobacterium microti, wherein said strain has integrated part or all of the RD1 region responsible for enhanced immunogenicity of the tubercle bacilli, especially the ESAT-6 and CFP-10 genes. These strains will be referred as the M. bovis BCG::RD1 or M. microti::RD1 strains and are useful as a new improved vaccine for

preventing tuberculosis and as a therapeutical product enhancing the stimulation of the immune system for the treatment of bladder cancer.
RE.CNT 11 THERE ARE 11 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 12 OF 24 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 9
AN 2004:64037 BIOSIS
DN PREV200400065524
TI CD8+-T-cell responses of mycobacterium-infected mice to a newly identified major histocompatibility complex class I-restricted epitope shared by proteins of the ESAT-6 family.
AU Majlessi, Laleh [Reprint Author]; Rojas, Marie-Jesus; Brodin, Priscille; Leclerc, Claude
CS Unite de Biologie des Regulations Immunitaires, Institut Pasteur, 25, Rue du Docteur Roux, 75724, Paris Cedex 15, France
lmajless@pasteur.fr
SO Infection and Immunity, (December 2003) Vol. 71, No. 12, pp. 7173-7177. print.
ISSN: 0019-9567 (ISSN print).
DT Article
LA English
ED Entered STN: 28 Jan 2004
Last Updated on STN: 28 Jan 2004
AB Here we describe the identification of a new CD8+-T-cell epitope, the GYAGTLQSL nonamer, shared by the TB10.3 and TB10.4 proteins of the Mycobacterium tuberculosis ESAT-6 family. Cytotoxic T cells from mycobacterium-infected mice efficiently recognized this epitope. GYAGTLQSL-specific T-cell hybridomas, which were able to recognize Mycobacterium bovis BCG-infected macrophages, were generated and now allow investigation of mycobacterial-antigen processing through the major histocompatibility complex class I pathway.

L14 ANSWER 13 OF 24 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 10
AN 2003:106356 BIOSIS
DN PREV200300106356
TI The shift of Th1 to Th2 immunodominance associated with the chronicity of Mycobacterium bovis bacille Calmette-Guerin infection does not affect the memory response.
AU Jiao, Xinan; Lo-Man, Richard; Winter, Nathalie; Deriaud, Edith; Gicquel, Brigitte; Leclerc, Claude [Reprint Author]
CS Unite de Biologie des Regulations Immunitaires, Institut Pasteur, 75015, Paris, France
cleclerc@pasteur.fr
SO Journal of Immunology, (February 1 2003) Vol. 170, No. 3, pp. 1392-1398. print.
ISSN: 0022-1767 (ISSN print).
DT Article
LA English
ED Entered STN: 19 Feb 2003
Last Updated on STN: 19 Feb 2003
AB In the present study we investigated the shaping and evolution of the immunodominance of the T cell response during a chronic mycobacterial infection. Using a recombinant bacille Calmette-Guerin expressing a reporter Ag, the Escherichia coli MalE protein, we analyzed the peptide specificity and the cytokine profile of the T cell response to the reporter Ag by ELISPOT. During the early steps of infection, the T cell response was focused on two dominant MalE epitopes and was characterized by a pure IFN-gamma response. Then, in the course of infection the initial IFN-gamma response to these two epitopes shifted to a mixed IFN-gamma/IL-4 response. At the same time, the peptide specificity of the T cell response was broadened to two additional MalE epitopes characterized by a unique IL-4 response resulting in the establishment of

a dominant IL-4 response to the MalE protein at 16 wk postinfection. However, this phenomenon did not impair the outcome of a predominant IFN-gamma response upon subsequent MalE recall in vivo performed in the presence of CFA, a Th1-driving adjuvant. These results indicate that the Th2 nature of the immune response established during a chronic infection, which most likely reflects regulatory mechanisms to allow the return to T cell homeostasis, does not shape the Th1/Th2 nature of the memory response.

L14 ANSWER 14 OF 24 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 11
AN 2003:249243 BIOSIS
DN PREV200300249243
TI Recombinant BCG exporting ESAT-6 confers enhanced protection against tuberculosis.
AU Pym, Alexander S.; Brodin, Priscille; Majlessi, Laleh; Brosch, Roland; Demangel, Caroline; Williams, Ann; Griffiths, Karen E.; Marchal, Gilles; Leclerc, Claude; Cole, Stewart T. [Reprint Author]
CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, Paris, France
stcole@pasteur.fr
SO Nature Medicine, (May 2003) Vol. 9, No. 5, pp. 533-539. print.
ISSN: 1078-8956 (ISSN print).
DT Article
LA English
ED Entered STN: 28 May 2003
Last Updated on STN: 28 May 2003
AB The live tuberculosis vaccines Mycobacterium bovis BCG (bacille Calmette-Guerin) and Mycobacterium microti both lack the potent, secreted T-cell antigens ESAT-6 (6-kDa early secretory antigenic target) and CFP-10 (10-kDa culture filtrate protein). This is a result of independent deletions in the region of deletion-1 (RD1) locus, which is intact in virulent members of the Mycobacterium tuberculosis complex. To increase their immunogenicity and protective capacity, we complemented both vaccines with different constructs containing the esxA and esxB genes, which encode ESAT-6 and CFP-10 respectively, as well as a variable number of flanking genes. Only reintroduction of the complete locus, comprising at least 11 genes, led to full secretion of the antigens and resulted in specific ESAT-6-dependent immune responses; this suggests that the flanking genes encode a secretory apparatus. Mice and guinea pigs vaccinated with the recombinant strain BCG::RD1-2F9 were better protected against challenge with M. tuberculosis, showing less severe pathology and reduced dissemination of the pathogen, as compared with control animals immunized with BCG alone.

L14 ANSWER 15 OF 24 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 12
AN 2003:7060 BIOSIS
DN PREV200300007060
TI Dendritic cells are host cells for mycobacteria in vivo that trigger innate and acquired immunity.
AU Jiao, Xinan; Lo-Man, Richard; Guermonprez, Pierre; Fiette, Laurence; Deriaud, Edith; Burgaud, Sophie; Gicquel, Brigitte; Winter, Nathalie; Leclerc, Claude [Reprint Author]
CS Unite de Biologie des Regulations Immunitaires, Institut Pasteur, 25 rue du Docteur Roux, 75724, Paris Cedex, 15, France
cleclerc@pasteur.fr
SO Journal of Immunology, (February 1 2002) Vol. 168, No. 3, pp. 1294-1301. print.
ISSN: 0022-1767 (ISSN print).
DT Article
LA English
ED Entered STN: 18 Dec 2002
Last Updated on STN: 18 Dec 2002

AB In the present study, we investigated in vivo the infection and APC functions of dendritic cells (DC) and macrophages (Mvariant pihi) after administration of live mycobacteria to mice. Experiments were conducted with Mycobacterium bovis bacillus Calmette-Guerin (BCG) or a rBCG expressing a reporter Ag. Following infection of mice, DC and Mvariant pihi were purified and the presence of immunogenic peptide/MHC class II complexes was detected ex vivo on sorted cells, as was the secretion of IL-12 p40. We show in this study that DC is a host cell for mycobacteria, and we provide an in vivo detailed picture of the role of Mphi and DC in the mobilization of immunity during the early stages of a bacterial infection. Strikingly, BCG bacilli survive but remain stable in number in the DC leukocyte subset during the first 2 wk of infection. As Ag presentation by DC is rapidly lost, this suggests that DC may represent a hidden reservoir for mycobacteria.

L14 ANSWER 16 OF 24 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 13
AN 2002:195739 BIOSIS
DN PREV200200195739
TI Identification of T cell epitopes of MalE protein expressed by recombinant Mycobacterium bovis BCG.
AU Jiao Xin'an [Reprint author]; Lo-Man, Richard; Deriaud, Edith; Burgaud, Sophie; Gicquel, Brigitte; Winter, Nathalie; Leclerc, Claude; Liu Xiufan
CS Yangzhou University, Yangzhou, 225009, China
jiao@mail.yzu.edu.cn
SO Zhonghua Weishengwuxue He Mianyixue Zazhi, (January, 2002) Vol. 22, No. 1, pp. 53-57. print.
CODEN: ZWMZDP. ISSN: 0254-5101.
DT Article
LA Chinese
ED Entered STN: 13 Mar 2002
Last Updated on STN: 13 Mar 2002
AB Objective: To identify T cell MalE epitopes protein by recombinant Mycobacterium bovis BCG (rBCG.MalE). Methods: The epitope repertoire of expressed MalE was analyzed in vitro by antigen presentation assay using dendritic cells as APC pulsed with rBCG.MalE, BCG.wt or purified MalE protein, and further analyzed in vivo or in vitro using T cell proliferation assay, IFN-gamma-ELISPOT and epitope mapping from immunized mice. Results: rBCG.MalE functionally expressed all 4 H-2d restricted T cell epitopes of native MalE. Conclusion: The p68-82 of expressed MalE was immunodominant epitope and the others were subdominant epitopes.

L14 ANSWER 17 OF 24 CAPLUS COPYRIGHT 2007 ACS on STN
AN 2005:341860 CAPLUS
DN 142:372453
TI Infection of dendritic cells by recombinant BCG
AU Jiao, Xin-an; Richard, Lo-Man; Guermonprez, Pierre; Derlaud, Edith; Gicquel, Brigitte; Winter, Nathalie; Leclerc, Claude; Liu, Xiu-fan
CS Key Lab of Ani Infec Dis, MOA, Yangzhou Univ., Yangzhou, 225009, Peop. Rep. China
SO Yangzhou Daxue Xuebao, Nongye Yu Shengming Kexueban (2002), 23(1), 1-5
CODEN: YDXNAX; ISSN: 1671-4652
PB Yangzhou Daxue Xuebao Bianjibu
DT Journal
LA Chinese
AB The infection and antigen-presenting cell functions of dendritic cells (DC) with recombinant BCG expressing MalE protein (rBCG · MalE) from Escherichia coli were investigated. In both in vitro and in vivo assays, DC can capture rBCG · MalE. 1%-2% Of the DC population was infected in vivo by the labeled rBCG · MalE through FACS anal., a similar percentage of MΦ population was also found to be

infected. It was shown that both DC and MΦ can phagocytose, process rBCG · MalE, and present T cell epitopes of MalE protein to specific T hybridoma FBU · B11 using in vitro antigen presentation assay. Following infection of mice, DC and MΦ were-purified and the presence of immunogenic peptide-MHC class II complexes could be detected ex vivo on sorted DC, but not on purified MΦ. These results suggest that DC play a pivotal role on the initiation of protective immune responses against mycobacteria.

L14 ANSWER 18 OF 24 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 14
AN 2000:279729 BIOSIS
DN PREV200000279729
TI Immune responses induced by recombinant BCG strains according to level of production of a foreign antigen: MalE.
AU Himmelrich, Hayo; Lo-Man, Richard; Winter, Nathalie; Guermonprez, Pierre; Sedlik, Christine; Rojas, Marie; Monnaie, Didier; Gheorghiu, Marina; Lagranderie, Micheline; Hofnung, Maurice; Gicquel, Brigitte; Clement, Jean-Marie; Leclerc, Claude [Reprint author]
CS Unite de Biologie des Regulations Immunitaires, CNRS URA 1444, Institut Pasteur, 25 Rue du Docteur Roux, 75724, Paris Cedex 15, France
SO Vaccine, (1 June, 2000) Vol. 18, No. 24, pp. 2636-2647. print.
CODEN: VACCDE. ISSN: 0264-410X.
DT Article
LA English
ED Entered STN: 6 Jul 2000
Last Updated on STN: 7 Jan 2002
AB A variety of viral, bacterial and parasitic antigens have been expressed in BCG and the capacity of these recombinant bacteria to induce immune responses has been well documented. However, little is known about the parameters influencing the induction of immune responses by recombinant BCG (rBCG), such as level of production and localization of the recombinant antigen. In the present study, we have constructed several rBCG strains expressing the malE gene from Escherichia coli which is either secreted or targeted to the cytoplasm or plasma membrane. Expression of malE was quantified by ELISA and localization was analyzed by flow cytometry. Even when using the same promoter, levels of cytoplasmic or membrane MalE production were far less than those from secreting strains using either mycobacterial or E. coli secretion signals. Stronger and more rapid immune responses were induced by rBCG strains with the highest levels of secreted MalE compared to cytoplasmic or membrane constructs, including both good humoral and proliferative responses in BALB/c, C57BL6 and even C3H mice, previously shown to be poor MalE responders. These results suggest that the levels of foreign antigen production play an important role in the induction of immune responses by rBCG strains.

L14 ANSWER 19 OF 24 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 15
AN 1997:392509 BIOSIS
DN PREV199799691712
TI Genetic control of antibody responses induced by recombinant Mycobacterium bovis BCG expressing a foreign antigen.
AU Lagranderie, Micheline; Lo-Man, Richard; Deriaud, Edith; Gicquel, Brigitte; Gheorghiu, Marina; Leclerc, Claude [Reprint author]
CS Biologie des Regulations Immunitaires, Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris, Cedex 15, France
SO Infection and Immunity, (1997) Vol. 65, No. 8, pp. 3057-3064.
CODEN: INFIBR. ISSN: 0019-9567.
DT Article
LA English
ED Entered STN: 10 Sep 1997
Last Updated on STN: 10 Sep 1997
AB Recombinant Mycobacterium bovis BCG expressing foreign antigens

represents a promising candidate for the development of future vaccines and was shown in several experimental models to induce protective immunity against bacteria) or parasitic infections. Innate resistance to BCG infection is under genetic control and could modify the immune responses induced against an antigen delivered by such engineered microorganisms. To investigate this question, we analyzed the immune responses of various inbred strains of mice to recombinant BCG expressing beta-galactosidase. These experiments demonstrated that BALB/c mice developed strong antibody responses against BCG expressing beta-galactosidase under the control of two different promoters. In contrast, C57BL/6, C3H, and CBA mice produced high anti-beta-galactosidase antibody titers only when immunized with recombinant BCG expressing beta-galactosidase under the control of the pblaF* promoter, which induced the production or high levels of this antigen. This difference in mouse responsiveness to recombinant BCG was not due to innate resistance to BCG infection, since similar immune responses were induced in Ity-r and Ity-s congenic strains of mice. In contrast, the analysis of anti-beta-galactosidase antibody responses of H-2 congenic mice in two different genetic backgrounds demonstrated that H-2 genes are involved in the immune responsiveness to beta-galactosidase delivered by recombinant BCG. Together, these results demonstrate that immune responses to an antigen delivered by recombinant BCG are under complex genetic influences which could play a crucial role in the efficiency of future recombinant BCG vaccines.

L14 ANSWER 20 OF 24 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 16

AN 1996:76917 BIOSIS

DN PREV199698649052

TI Comparison of immune responses of mice immunized with five different Mycobacterium bovis BCG vaccine strains.

AU Lagranderie, Micheline R. R.; Balazuc, Annie-Marie; Deriaud, Edith; Leclerc, Claude D.; Gheorghiu, Marina [Reprint author]

CS Lab. BCG, Inst. Pasteur, 25 rue du Dr. Roux, 75724 Paris Cedex 15, France

SO Infection and Immunity, (1996) Vol. 64, No. 1, pp. 1-9.
CODEN: INFIBR. ISSN: 0019-9567.

DT Article

LA English

ED Entered STN: 27 Feb 1996
Last Updated on STN: 27 Feb 1996

AB Among the various parameters which may contribute to Mycobacterium bovis BCG vaccination efficiency, the choice of the vaccine strain may play an important role. In the present study, we therefore compared the immunogenicity of five different BCG strains that are commonly used for BCG vaccine production (Glaxo 1077, Japanese 172, Pasteur 1173P2, Prague, and Russian strains). The comparison of the growth capacity of these BCG strains in BALB/c and C3H mice demonstrated that a great difference exists between the capacity of various BCG strains to multiply and persist in target organs. A much lower recovery of BCG could be shown in mice immunized with Prague and Japanese BCG strains. T-cell responses of BCG-immunized mice were also examined by analyzing T-cell proliferative responses, cytokine production, delayed-tvpe hypersensitivity responses, and cytotoxic activity. All these assays demonstrated that BCG immunization induced strong CD4+ T-cell responses, mostly of the Th1 type, as demonstrated by interleukin-2 and gamma interferon production. These studies also demonstrated that there are differences between BCG strains in stimulating these T-cell responses. A lack of induction of cytotoxic activity was observed following immunization with the Japanese strain. Lower anti-purified protein derivative antibody responses were also observed after intravenous or oral immunization with this BCG strain. Finally, the protective activity of these BCG strains was tested by measuring

the capacity of immunized mice to eliminate recombinant Pasteur and Japanese BCG strains which expressed beta-galactosidase. The results of these experiments clearly demonstrated that the Prague and Japanese strains were unable to protect mice against a second mycobacterial challenge whereas mice immunized with the Glaxo, Pasteur, or Russian strain eliminated the recombinant BCG very efficiently. Altogether, the results of the present study strongly support the view that there are considerable differences in the immunogenicity of various BCG vaccine strains and that these differences may play a major role in BCG vaccination efficiency.

L14 ANSWER 21 OF 24 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 17
AN 1995:271937 BIOSIS
DN PREV199598286237
TI Recombinant BCG strains expressing the SIV-mac251nef gene induce proliferative and CTL responses against nef synthetic peptides in mice.
AU Winter, Nathalie; Lagranderie, Micheline; Gangloff, Sophie; Leclerc, Claude; Gheorghiu, Marina; Gicquel, Brigitte [Reprint author]
CS Unite Genetique Mycobacterienne, Institut Pasteur, 28 Rue Dr. Roux, 75724 Paris, Cedex 15, France
SO Vaccine, (1995) Vol. 13, No. 5, pp. 471-478.
CODEN: VACCDE. ISSN: 0264-410X.
DT Article
LA English
ED Entered STN: 26 Jun 1995
Last Updated on STN: 26 Jun 1995
AB CTL responses are known to be important for the control of HIV and SIV infections. Such responses are targeted against various components of these viruses including regulatory proteins like Nef. The SIV-mac251nef gene was cloned in Mycobacterium bovis BCG under the control of P-AN, a promoter from Mycobacterium paratuberculosis. Nef was expressed as a fused polypeptide with ORF2, an open reading frame adjacent to P-AN. Mice inoculated with rBCG(SIV-mac251nef) exhibited proliferative and CD8+ cytotoxic T-cell (CTL) responses against several Nef synthetic peptides. A mapping of the epitopes recognized by CTLs revealed that the central region of Nef is mainly involved in responses. This region had already been demonstrated to induce CTLs in experimentally SIV-infected macaques as well as in HIV-infected individuals. These results demonstrate the feasibility of constructing BCG vaccine strains expressing nef for eliciting cytotoxic responses.

L14 ANSWER 22 OF 24 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 18
AN 1994:499640 BIOSIS
DN PREV199497512640
TI Mycobacterium bovis BCG priming induces a strong potentiation of the antibody response induced by recombinant BCG expressing a foreign antigen.
AU Gheorghiu, Marina; Lagranderie, Micheline R. R.; Gicquel, Brigitte M. E.; Leclerc, Claude D. [Reprint author]
CS Biol. Regulations Immunitaires, Inst. Pasteur, 25 rue du Docteur Roux, 75724 Paris Cedex 15, France
SO Infection and Immunity, (1994) Vol. 62, No. 10, pp. 4287-4295.
CODEN: INFIBR. ISSN: 0019-9567.
DT Article
LA English
ED Entered STN: 28 Nov 1994
Last Updated on STN: 28 Nov 1994
AB Several recent studies have demonstrated that strong cellular or humoral immune responses can be induced against foreign antigens expressed by recombinant Mycobacterium bovis BCG. It has therefore been suggested that BCG could represent one of the best candidate vectors for live recombinant vaccines. However, a large percentage of the

human population has been immunized by BCG, and this priming could modify the immune response to future recombinant BCG vaccines. In the present study, we have therefore compared the immune responses induced in naive and BCG-primed mice by two recombinant BCG vaccines expressing either beta-galactosidase or human immunodeficiency virus type 1 Nef antigens. Our results demonstrated that BCG priming limits the growth of recombinant BCG in mouse spleen or lymph nodes. This reduction in BCG growth was associated with decreased proliferative responses against Nef or beta-galactosidase antigens. This suppression, however, never exceeded 50%. Interestingly, in contrast to these reduced T-cell responses, BCG-primed mice developed high levels of anti-beta-galactosidase antibodies after immunization with recombinant BCG expressing this antigen. This stimulation of antibody responses was not due to polyclonal stimulation or to a nonspecific adjuvant effect of BCG . The isotypic patterns of anti-beta-galactosidase antibody responses induced by the recombinant BCG were similar in naive and BCG-primed mice. These results indicate that priming with BCG will not be a limitation for the use of recombinant BCC vaccines in humans.

L14 ANSWER 23 OF 24 CAPLUS COPYRIGHT 2007 ACS on STN
AN 1992:590061 CAPLUS
DN 117:190061
TI Expression of the HIV-1 nef gene in Mycobacterium bovis BCG and induction of a T-cell response against the Nef antigen
AU Winter, Nathalie; Lagranderie, Micheline; Rauzier, Jean; Timm, Juliano; Leclerc, Claude; Gheorghiu, Marina; Gicquel, Brigitte; Guy, Bruno; Kieny, Marie Paule
CS Unite Genie Microbiol., Inst. Pasteur, Paris, 75015, Fr.
SO Vaccines 92: Mod. Approaches New Vaccines Incl. Prev. AIDS [Annu. Meet.], 9th (1992), 373-8. Editor(s): Brown, Fred. Publisher: Cold Spring Harbor Lab. Press, Cold Spring Harbor, N. Y.
CODEN: 57WXAL
DT Conference
LA English
AB A high level of expression of the HIV-1 virus nef regulatory gene in M. bovis BCG was obtained using a heat-shock promoter from Staphylococcus albus. Lymph node cells from mice immunized with BCG-nef recombinant proliferated vigorously in response to the Nef protein, which might be a suitable target in a vaccine strategy. This 1st report of a T-cell response measured by a proliferation test suggests that BCG recombinants may be used to immunize against pathogen in which a T-cell mediated immune response is important for protection.

L14 ANSWER 24 OF 24 CAPLUS COPYRIGHT 2007 ACS on STN
AN 1993:647293 CAPLUS
DN 119:247293
TI Expression of foreign antigens in Mycobacterium bovis BCG recombinant strains
AU Winter, Nathalie; Murray, Alan; Lagranderie, Micheline; Rauzier, Jean; Timm, Juliano; Leclerc, Claude; Guy, Bruno; Keiny, Marie Paule; Gheorghiu, Marina; Gicquel, Brigitte
CS Unite Genie Microbiol., Inst. Pasteur, Paris, Fr.
SO Retroviruses Hum. A.I.D.S. Relat. Anim. Dis., Colloq. "Cent Gardes", 6th (1991), 117-22. Editor(s): Girard, Marc; Valette, Louis. Publisher: Fond. Marcel Merieux, Lyon, Fr.
CODEN: 59HSAD
DT Conference; General Review
LA English
AB A review, with 14 refs., suggesting that BCG seems to be a promising vector for the construction of polyvalent recombinant vaccines able to induce cellular and humoral immune responses simultaneously. Other HIV and SIV antigens can be cloned in BCG, either on

replicative or integrative vectors and using various expression cassettes allowing the expression of intracellular or secreted products when BCG grows intracellularly. The capability to insert numerous whole antigens in BCG might allow the induction of immune responses against several proteins, even those containing different variable motifs.

=> s (bcg or microti) and (RD1-2F9)
L15 8 (BCG OR MICROTI) AND (RD1-2F9)

=> dup rem l15
PROCESSING COMPLETED FOR L15
L16 2 DUP REM L15 (6 DUPLICATES REMOVED)

=> d bib ab 1-
YOU HAVE REQUESTED DATA FROM 2 ANSWERS - CONTINUE? Y/(N):y

L16 ANSWER 1 OF 2 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 1
AN 2004:326990 BIOSIS
DN PREV200400328635
TI Enhanced protection against tuberculosis by vaccination with recombinant Mycobacterium microti vaccine that induces T cell immunity against region of difference 1 antigens.
AU Brodin, Priscille; Majlessi, Laleh; Brosch, Roland; Smith, Debbie; Bancroft, Gregory; Clark, Simon; Williams, Ann; Leclerc, Claude; Cole, Stewart T. [Reprint Author]
CS Unite Genet Mol Bacterienne, Inst Pasteur, 28 Rue Docteur Roux, F-75724, Paris, 15, France
stcole@pasteur.fr
SO Journal of Infectious Diseases, (July 1 2004) Vol. 190, No. 1, pp. 115-122. print.
CODEN: JIDIAQ. ISSN: 0022-1899.
DT Article
LA English
ED Entered STN: 29 Jul 2004
Last Updated on STN: 29 Jul 2004
AB Mycobacterium microti, the vole bacillus, which was used as a live vaccine against tuberculosis until the 1970s, confers the same protection in humans as does Mycobacterium bovis bacille Calmette-Guerin (BCG). However, because the efficacy of the BCG vaccine varies considerably, we have tried to develop a better vaccine by reintroducing into M. microti the complete region of difference 1 (RD1), which is required for secretion of the potent T cell antigens early secreted antigen target (ESAT)-6 and culture filtrate protein (CFP)-10. The resultant recombinant strain, M. microti OV254:: RD1-2F9, induced specific ESAT-6 and CFP-10 immune responses in mice with CD8+ T lymphocytes that had strong expression of the CD44hi activation marker. This vaccine also displayed better efficacy against disseminated disease in the mouse and the guinea pig models of tuberculosis than was seen in animals vaccinated with M. microti alone or with BCG. The M. microti OV254::RD1 -2F9 vaccine was less virulent and persistent in mice and than was BCG::RD1-2F9 may represent a safer alternative to BCG::RD1-2F9.

L16 ANSWER 2 OF 2 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 2
AN 2003:249243 BIOSIS
DN PREV200300249243
TI Recombinant BCG exporting ESAT-6 confers enhanced protection against tuberculosis.
AU Pym, Alexander S.; Brodin, Priscille; Majlessi, Laleh; Brosch, Roland;

Demangel, Caroline; Williams, Ann; Griffiths, Karen E.; Marchal, Gilles; Leclerc, Claude; Cole, Stewart T. [Reprint Author]

CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, Paris, France
stcole@pasteur.fr

SO Nature Medicine, (May 2003) Vol. 9, No. 5, pp. 533-539. print.
ISSN: 1078-8956 (ISSN print).

DT Article

LA English

ED Entered STN: 28 May 2003
Last Updated on STN: 28 May 2003

AB The live tuberculosis vaccines Mycobacterium bovis BCG (bacille Calmette-Guerin) and Mycobacterium microti both lack the potent, secreted T-cell antigens ESAT-6 (6-kDa early secretory antigenic target) and CFP-10 (10-kDa culture filtrate protein). This is a result of independent deletions in the region of deletion-1 (RD1) locus, which is intact in virulent members of the Mycobacterium tuberculosis complex. To increase their immunogenicity and protective capacity, we complemented both vaccines with different constructs containing the esxA and esxB genes, which encode ESAT-6 and CFP-10 respectively, as well as a variable number of flanking genes. Only reintroduction of the complete locus, comprising at least 11 genes, led to full secretion of the antigens and resulted in specific ESAT-6-dependent immune responses; this suggests that the flanking genes encode a secretory apparatus. Mice and guinea pigs vaccinated with the recombinant strain BCG::RD1-2F9 were better protected against challenge with M. tuberculosis, showing less severe pathology and reduced dissemination of the pathogen, as compared with control animals immunized with BCG alone.

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=> s (mycobact?) and (RD1-2F9)
L17          8 (MYCOBACT?) AND (RD1-2F9)

=> dup rem l17
PROCESSING COMPLETED FOR L17
L18           2 DUP REM L17 (6 DUPLICATES REMOVED)

=> d bib ab 1-
YOU HAVE REQUESTED DATA FROM 2 ANSWERS - CONTINUE? Y/(N):y
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L18 ANSWER 1 OF 2 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 1

AN 2004:326990 BIOSIS

DN PREV200400328635

TI Enhanced protection against tuberculosis by vaccination with recombinant Mycobacterium microti vaccine that induces T cell immunity against region of difference 1 antigens.

AU Brodin, Priscille; Majlessi, Laleh; Brosch, Roland; Smith, Debbie; Bancroft, Gregory; Clark, Simon; Williams, Ann; Leclerc, Claude; Cole, Stewart T. [Reprint Author]

CS Unite Genet Mol Bacterienne, Inst Pasteur, 28 Rue Docteur Roux, F-75724, Paris, 15, France
stcole@pasteur.fr

SO Journal of Infectious Diseases, (July 1 2004) Vol. 190, No. 1, pp. 115-122. print.
CODEN: JIDIAQ. ISSN: 0022-1899.

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LA English

ED Entered STN: 29 Jul 2004
Last Updated on STN: 29 Jul 2004

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varies considerably, we have tried to develop a better vaccine by reintroducing into M. microti the complete region of difference 1 (RD1), which is required for secretion of the potent T cell antigens early secreted antigen target (ESAT)-6 and culture filtrate protein (CFP)-10. The resultant recombinant strain, M. microti OV254::RD1-2F9, induced specific ESAT-6 and CFP-10 immune responses in mice with CD8+ T lymphocytes that had strong expression of the CD44hi activation marker. This vaccine also displayed better efficacy against disseminated disease in the mouse and the guinea pig models of tuberculosis than was seen in animals vaccinated with M. microti alone or with BCG. The M. microti OV254::RD1-2F9 vaccine was less virulent and persistent in mice and than was BCG::RD1-2F9 may represent a safer alternative to BCG::RD1-2F9.

L18 ANSWER 2 OF 2 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 2
AN 2003:249243 BIOSIS
DN PREV200300249243
TI Recombinant BCG exporting ESAT-6 confers enhanced protection against tuberculosis.
AU Pym, Alexander S.; Brodin, Priscille; Majlessi, Laleh; Brosch, Roland; Demangel, Caroline; Williams, Ann; Griffiths, Karen E.; Marchal, Gilles; Leclerc, Claude; Cole, Stewart T. [Reprint Author]
CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, Paris, France
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=> s (RD1-2F9)
L19          8 (RD1-2F9)

=> dup rem l19
PROCESSING COMPLETED FOR L19
L20            2 DUP REM L19 (6 DUPLICATES REMOVED)

=> d bib ab 1-
YOU HAVE REQUESTED DATA FROM 2 ANSWERS - CONTINUE? Y/(N):y

L20  ANSWER 1 OF 2  BIOSIS  COPYRIGHT (c) 2007 The Thomson Corporation  on STN
     DUPLICATE 1
```

AN 2004:326990 BIOSIS
DN PREV200400328635
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AU Brodin, Priscille; Majlessi, Laleh; Brosch, Roland; Smith, Debbie; Bancroft, Gregory; Clark, Simon; Williams, Ann; Leclerc, Claude; Cole, Stewart T. [Reprint Author]
CS Unite Genet Mol Bacterienne, Inst Pasteur, 28 Rue Docteur Roux, F-75724, Paris, 15, France
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L20 ANSWER 2 OF 2 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 2
AN 2003:249243 BIOSIS
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AU Pym, Alexander S.; Brodin, Priscille; Majlessi, Laleh; Brosch, Roland; Demangel, Caroline; Williams, Ann; Griffiths, Karen E.; Marchal, Gilles; Leclerc, Claude; Cole, Stewart T. [Reprint Author]
CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, Paris, France
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DT Article
LA English
ED Entered STN: 28 May 2003
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AB The live tuberculosis vaccines Mycobacterium bovis BCG (bacille Calmette-Guerin) and Mycobacterium microti both lack the potent, secreted T-cell antigens ESAT-6 (6-kDa early secretory antigenic target) and CFP-10 (10-kDa culture filtrate protein). This is a result of independent deletions in the region of deletion-1 (RD1) locus, which is intact in virulent members of the Mycobacterium tuberculosis complex. To increase their immunogenicity and protective capacity, we complemented both vaccines with different constructs containing the esxA and esxB genes, which encode ESAT-6 and CFP-10 respectively, as well as a variable number of flanking genes. Only reintroduction of the complete locus, comprising

at least 11 genes, led to full secretion of the antigens and resulted in specific ESAT-6-dependent immune responses; this suggests that the flanking genes encode a secretory apparatus. Mice and guinea pigs vaccinated with the recombinant strain BCG::RD1-2F9 were better protected against challenge with M. tuberculosis, showing less severe pathology and reduced dissemination of the pathogen, as compared with control animals immunized with BCG alone.